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# Nitrification rates in a reversed-flow, spouted-bed, bioreactor applied to recirculating aquaculture systems

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# NITRIFICATION RATES IN A REVERSED-FLOW, SPOUTED-BED, BIOREACTOR APPLIED TO RECIRCULATING AQUACULTURE SYSTEMS

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## ABSTRACT

The effects of media selection and organic loading on nitrification rates in a reversed-flow, three-phase, spouted-bed, bioreactor with draft-tube (A-1 Aquaculture Continuous-Cleaning Multifunctional Biofilter or CCMB) were studied. Experiments were conducted on three identical recirculating aquaculture systems (RAS) each having a CCMB unit with a unique plastic pelletized media and operated over five successive trials with varying components and operating conditions. Based upon organic loading, three of the five trials were grouped into two organic loading regimes of approximately 91 mg/L COD and 149 mg/L COD.

Comparing nitrification rates against the three media types by organic loading showed that media selection had a significant ($p<0.05$) impact on nitrification performance. At both levels of organic loading, the nitrification rates of Media 1 outperformed Media 2 and Media 3. The differences between the Media 1 rates and those of Media 2 and Media 3 were greater at the lower organic loading than at the higher loading, for nitratation than for nitritation, and for areal comparisons than for volumetric comparisons.

Comparing nitritation and nitratation rates against organic loading for each media type showed that organic loading had little impact on nitrification. A barely significant ($p<0.05$) difference between nitrification rates at the two organic loadings was observed only for Media 1 nitratation, where the nitratation rate was greater at the lower organic loading.

Throughout all five trials, the CCMB demonstrated the ability to successfully nitrify over organic levels ranging from 13.5 to 205.3 mg/L COD and without showing

any signs of biofouling or other problems associated with traditional fixed-film nitrification systems. Media 1 achieved the highest mean nitrification rates during all trials with an average concentration-normalized volumetric nitritation rate of 223 g TAN/day-$m^3$ (0.0139 lb TAN/day-$ft^3$) and maximum of 254 g TAN/day-$m^3$ (0.0159 lb TAN/day-$ft^3$) observed during Trial 5, which had both the highest organic loading and flow rates of all trials.

# CHAPTER 1

# INTRODUCTION

In late 1994, A-1 Aquaculture of Bush, LA requested that the Louisiana State University (LSU) Department of Biological and Agricultural Engineering evaluate a prototype biofilter for nitrification in aquaculture systems referred to as the Continuous-Cleaning Multifunctional Biofilter (CCMB). The unit was placed into a recirculating aquaculture system (RAS), from which the results of a preliminary nitrification capacity evaluation looked very promising (Lawson et al., 1996).

In biological reactor (bioreactor) terms, the unit can be described as a three-phase, reversed-flow, spouted-bed, bioreactor with draft-tube utilizing fixed-film nitrifying organisms grown on pelletized low density plastic media. The term "three-phase" is assigned to reactors containing the three phases of solid (biofilm covered media pellets), liquid (culture water), and gas (air). In wastewater-treatment terms, the unit might be described as a dynamic-bed nitrification filter or moving-bed biofilm treatment system. While in aquaculture terms, if not referred to as the CCMB the unit might be called by some a "floating bead filter," though that term is generally reserved for static-bed floating plastic pellet (bead) units also called expandable granular biofilters (EGBs) used to simultaneously capture solids and perform nitrification in a pressurized variable-flow filter and should not be confused with dynamic systems containing floating plastic pellet media such as the CCMB (Malone and Beecher, 2000). However, it should be noted that the CCMB does possess an alternate mode of operation, not addressed in this study, whereby the media is held static and the unit performs the same operations as the static "floating bead filter." The notable difference is that the CCMB

in “static mode” requires complicated backflushing, retains its full flow rate during use, and is not contained in a pressurized vessel like the “floating bead filter,” which slowly clogs during use (Scott et al., 1997).

### 1.1 Objectives

As a prelude to performing CCMB process optimization research, the goal of this study was to establish the potential for significant alterations in CCMB performance from basic unit design and operational criteria. Specifically, the objectives of the study were:

- to determine if media selection is significant to CCMB performance; and
- to determine if organic loading has a significant impact on CCMB performance; and while meeting both of the above stated objectives
- to calculate the nitrification rates of the CCMB so that it could be sized to future aquaculture systems.

### 1.2 Approach

Three identical RAS were constructed at the Louisiana State University Ben Hur Aquacultural Research Facility within an indoor laboratory. To meet the objectives of this study, each system utilized a different plastic biofilm media, while the solids removal scheme was altered over 3 steady-state trials to achieve increasing organic loads against which to evaluate the nitrification rates of each system. The objectives were met by comparing calculated steady-state nitrification rates between media types and solids loading levels.

# CHAPTER 2

# LITERATURE REVIEW

Here at the dawn of the new millennium we are aiming new diagnostic tools such as fluorescent *in situ* hybridization and oligonucleotide probes at our microbial systems and the knowledge we are gaining are creating more questions than answers. We have made great strides in our understanding of these very complicated systems and yet, the definition of a true “theoretical biology” continues to elude us. Missing from the plethora of quantitative models that are piecemealed together under today's definition of "theoretical biology" are the relational aspects of biological processes that would be expected from a "physical theory" (Welch, 1993).

The challenge to biological engineering is to analyze the processes of biology even when they are not fully understood by an exact science so that we can design and operate them to our benefit. When possible and practical we continue to extend our study of biological processes beyond the qualitative in order to develop quantitative mathematical representations of the chemical reactors, control systems, and mass-transfer operations contained within living systems. The fundamental concepts of both biology and engineering serve as our guide.

## 2.1 The Nitrogen Cycle

Nitrogen exists in all living things. As ammonia ($NH_3$), nitrogen enables the production of amino acids, the essential building blocks of nucleic acids and proteins (Painter, 1970; McGilvery, 1975). Though most never think about it, terrestrial life practically swims in a sea of nitrogen, as it comprises 75.51% by mass (0.7808 mol fraction) of the Earth's atmosphere and 3.1% of the human body (McQuarrie and Rock, 1991).

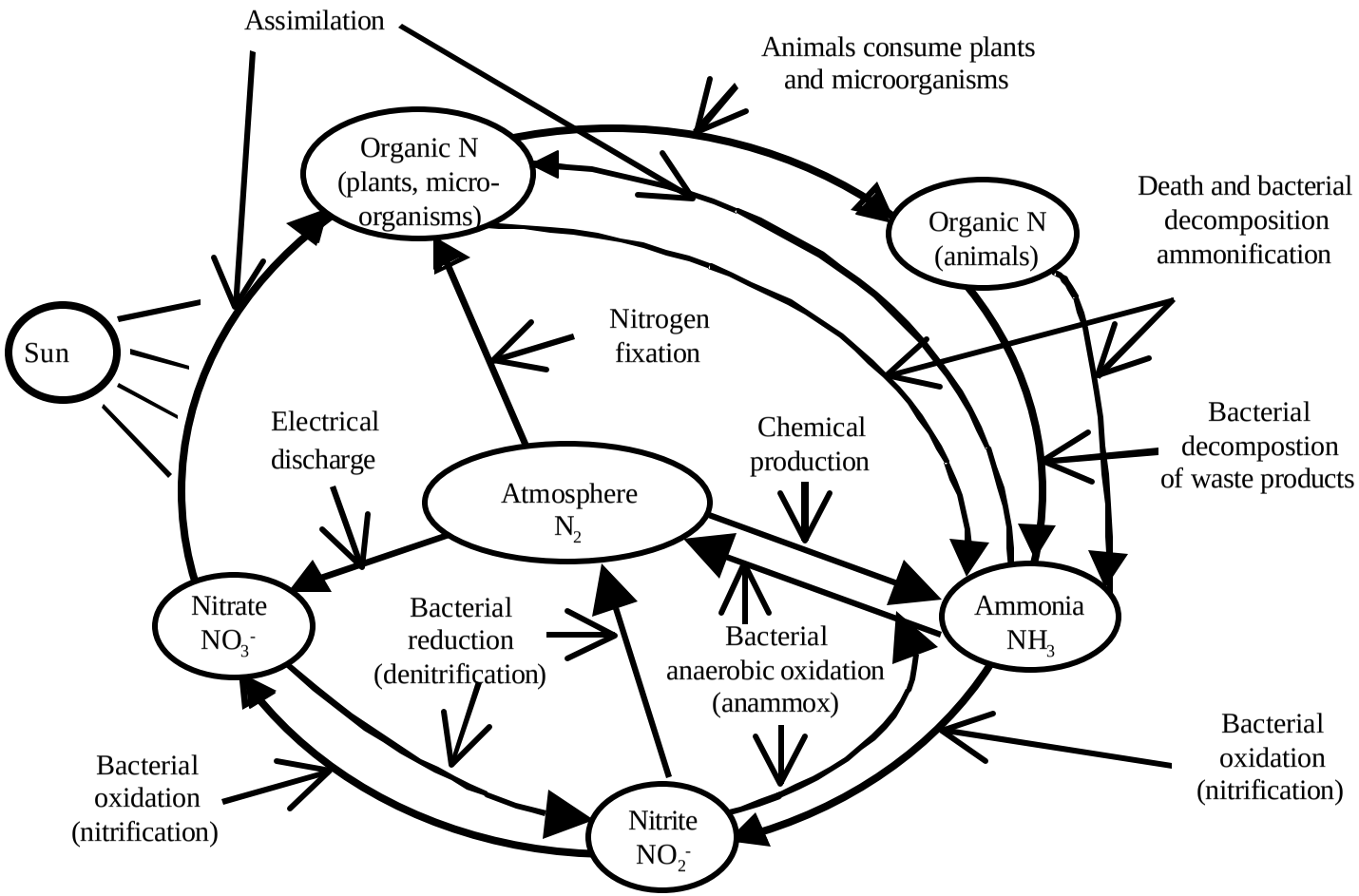


Figure 2.1-1. Nitrogen conversions. (adapted from Tchobanoglous and Schroeder, 1987; Loosdrecht and Jetten, 1998)

Though there is abundant nitrogen in our surroundings, in certain forms and in certain environments, nitrogen can cause problems.

## 2.2 Importance of Nitrogen Control

There are three primary reasons that nitrogen is of concern in aquatic systems (Jorgensen and Halling-Sorensen, 1993). First, nitrogen contributes to eutrophication (Loehr, 1984; Blackall and Burrell, 1999). Second, the oxidation of organic and reduced forms of inorganic nitrogen by microorganisms creates an oxygen ($O_2$) demand (Loehr, 1984). Third, certain forms of nitrogen are directly toxic to many aquatic organisms (Loehr, 1984; Russo, 1985; Boyd, 1990; Lawson, 1995). Other reasons include methaemoglobinaemia in infants (Blackall and Burrell, 1999) and the related brown blood disease in fish (Lawson, 1995), $N_2$ gas supersaturation (Spotte, 1979), and increased chlorine ($Cl_2$) disinfection demands where $NH_3$-containing sources are used for potable water (Loehr, 1984).

### 2.2.1 Eutrophication

Eutrophication is a term used to describe the increased fertility of natural waters exceeding the growth limiting concentrations of photosynthetic cyanobacteria, eukaryotic algae, and/or macrophytes (Blackhall and Burrell, 1999). The increased growth of photosynthetic organisms (often referred to as “algal blooms”) has several negative aesthetic and life-supporting consequences (Jorgensen and Halling-Sorensen, 1993; Blackhall and Burrell, 1999). The most common consequence of algal blooms are fish kills (Boyd, 1990) resulting from $O_2$ depletion by the night-time respiration of photosynthetic organisms (Wilson et al., 1971) and the breakdown of algal biomass by chemoheterotrophic bacteria (Boyd, 1990). Fish kills can also be caused by the release of toxins produced by certain cyanobacteria (Codd, 1995). Through ingestion or even

contact these toxins can cause an array of health problems in animals and have reportedly killed animals as large as cattle (Codd, 1995). Though "natural eutrophication" does occur, it is normally much more subtle than the literally lethal population explosions resulting from human activity termed "cultural eutrophication" (Wilson et al., 1971).

2.2.2 Nitrogenous Oxygen Demand (NOD)

The oxidative bacterial decomposition of nitrogenous compounds to $NO_3^-$ removes a tremendous amount of $O_2$ from aquatic systems. The $O_2$ deficit created can seriously alter aquatic system dynamics and the $NO_3^-$ produced can be a significant accelerant for eutrophication (Loehr, 1984).

2.2.3 Direct Toxicity

The three forms of nitrogen that can be directly toxic to aquatic organisms are $NH_3$, $NO_2^-$, and to a lesser extent $NO_3^-$ (Russo, 1985; Boyd, 1990; Lawson, 1995). Though in general, aquatic organisms are more sensitive to $NH_3$, with some species showing chronic impairment at concentrations as low as 0.02 mg/L as $NH_3$-N (Lawson, 1995).

Ammonia concentrations in water are measured in mg/L of total ammonia nitrogen (TAN). It is called total ammonia nitrogen because it is actually a measurement of both the ionized ($NH_4^+$) and unionized ($NH_3$) forms of ammonia expressed as nitrogen. The two species are in equilibrium as shown by Equation 2.2.3-1.

$$NH_3 + H^+ \leftrightarrows NH_4^+ \qquad (2.2.3\text{-}1)$$

The fraction of TAN that exists in the $NH_3$-N form is termed the mole fraction of $NH_3$-N and can be estimated with the following equation (Lawson, 1995).

$$mf_{(NH_3-N)} = \frac{1}{1+10^{(10.068-0.033 \cdot T-pH)}} \qquad (2.2.3\text{-}2)$$

where

| | | | |
|---|---|---|---|
| $mf_{(NH3-N)}$ | = | mole fraction of $NH_3$-N | (decimal fraction) |
| T | = | temperature | (°C) |
| pH | = | pH | (unitless) |

At typical warmwater aquaculture conditions of 7.5 pH and 28 °C, Equation 2.2.3-2 shows the mole fraction of $NH_3$ -N to be only 0.02. Because this value is so small, TAN can be used in place of $NH_4^+$-N for simplicity (Lawson, 1995). The recommended safe concentration of TAN and $NO_2^-$-N for long-term exposure in average freshwater fish aquaculture grow-out systems is 1.0 mg/L (Lawson, 1995; Malone and DeLosReyes, 1997).

**2.3 Nitrification**

First documented in 1887 (Peters and Foley, 1983), the biological process whereby toxic ammonia ($NH_3$) is converted to non-toxic nitrate ($NO_3^-$) through the action of autotrophic nitrifying bacteria is termed nitrification. The nitrification process is of commercial concern to both the aquaculture and wastewater industries since both have a vested interest in the wellbeing of aquatic organisms sensitive to low $NH_3$ levels (Rand & Petrocelli, 1985; Boyd, 1990; Lawson, 1995).

It is generally accepted in the historical literature that the oxidation of $NH_3$ and $NO_2^-$ are brought about, respectively, by the bacterial genera *Nitrosomonas* and *Nitrobacter* in a two-stage process as first reported by Winogradsky in 1892 (Hochheimer, 1990; Brock et al., 1994). During the first stage the ionized form of ammonia ($NH_4^+$) is converted to nitrite ($NO_2^-$) by the genus *Nitrosomonas*. The second stage follows with the genus *Nitrobacter* converting $NO_2^-$ to $NO_3^-$. Equations 2.3-3 and 2.3-4 illustrate the general stoichiometric reactions for stages 1 and 2 (USEPA, 1975; Tchobanoglous, 1979; WPCF, 1983), sometimes referred to respectively as nitrification

and nitrafication, or nitritation and nitratation (Mauret et al., 1996). The term nitrification is generally used to describe both stages (Brock et al., 1994).

While it has been suspected for some time that other organisms may be responsible for nitrification (Knowles et al., 1964; Painter, 1970; Watson et al., 1981), the results of recent microbial studies have made the identification of the predominant microorganisms a topic of much debate (Wagner et al., 1993; Wagner et al., 1996; Prinèiè et. al, 1998; Biesterfeld, 1999; Jones et al., 2000). Primarily through the use of modern methods of detection such as fluorescent *in situ* hybridization and oligonucleotide probes, researchers are finding that *Nitrosomonas* or *Nitrobacter* are either not present, or not the predominant nitrifying organisms (Prinèiè et. al, 1998; Jones et al., 2000; Biesterfeld, 2001). The current explanation for the difference between the microorganisms identified and those present is that the laboratory culture techniques are biased towards *Nitrosomonas* and *Nitrobacter* (Wagner et al., 1993).

As the debate has not yet been resolved and much of the historical literature is based on the results of research performed on systems in which no attempts were made to identify the nitrifying organisms, the specific or mix of microorganisms responsible for nitrification will continue to be referred to as *Nitrosomonas* and *Nitrobacter*.

$$NH_4^+ + 1.5\ O_2 \rightarrow 2\ H^+ + H_2O + NO_2^- \qquad (2.3\text{-}1)$$

$$NO_2^- + 0.5\ O_2 \rightarrow NO_3^- \qquad (2.3\text{-}2)$$

Combining Equations 2.3-1 and 2.3-2, equation 2.3-3 shows the complete oxidation of $NH_4^+$ to $NO_3^-$.

$$NH_4^+ + 2\ O_2 \rightarrow NO_3^- + 2\ H^+ + H_2O \qquad (2.3\text{-}3)$$

The same steps are shown with the addition of cell growth in Equations 2.3-4 through 2.3-6 below where $C_5H_7O_2N$ represents bacterial biomass (Grady et al., 1999).

$$55\ NH_4^+ + 76\ O_2 + 109\ HCO_3^- \rightarrow C_5H_7O_2N + 54\ NO_2^- + 57\ H_2O + 104\ H_2CO_3 \quad (2.3\text{-}4)$$

$$400\ NO_2^- + NH_4^+ + 4\ H_2CO_3 + HCO_3^- + 195\ O_2 \rightarrow C_5H_7O_2N + 3\ H_2O + 400\ NO_3^- \quad (2.3\text{-}5)$$

Combining Equations 2.3-4 and 2.3-5 and simplifying yields Equation 2.3-6.

$$NH_4^+ + 1.83\ O_2 + 1.98\ HCO_3^- \rightarrow$$

$$0.021\ C_5H_7O_2N + 1.041\ H_2O + 0.98\ NO_3^- + 1.88\ H_2CO_3 \quad (2.3\text{-}6)$$

In Equation 2.3-1 N is the electron donor and O the electron acceptor with a total of 6 electrons ($e^-$) transferred as 1 mole of $NH_4^+$ is oxidized to $NO_2^-$. Again, N is the electron donor and O the electron acceptor in Equation 2.3-2 when 2 $e^-$ are transferred as 1 mole of $NO_2^-$ is oxidized to $NO_3^-$. The combined Equation 2.3-3 agrees that 8 $e^-$ are transferred in the complete oxidation of $NH_4^+$ to $NO_3^-$. Chemical Oxygen Demand (COD) can also be used as a measure of the energy transfer in a reaction or the energy available in a substrate.

Since bacterial biomass ($C_5H_7O_2N$) also incorporates nitrogen, we know that nitrogen is not only used as the electron donor (Equations 2.3-1 through 2.3-3), but that it is also used for cell growth as shown in Equations 2.3-4 through 2.3-6 (Grady and Lim, 1980).

From equation 2.3-6 we can determine that for each mg of $NH_4^+$-N oxidized to $NO_3^-$-N, the combined reactions require approximately 4.27 mg $O_2$ and 8.81 mg $HCO_3^-$ (15.0 mg as $CaCO_3$). The 0.24 mg $O_2$ difference between Equations 2.3-3 and 2.3-6 are due to the formation of new biomass and represent the true growth yields ($Y_A$) of 0.212 mg biomass COD formed/mg N oxidized by *Nitrosomonas* and 0.0286 mg biomass

COD formed/mg N oxidized by *Nitrobacter* (Grady et al., 1999). It should be noted that the yield of nitrifiers is less than half that of most heterotrophic bacteria.

**2.4 Microbial Kinetics**

Purification of water by bacteria was first patented in 1865 and by 1887 engineering studies were underway to optimize the process. Experimental results were quickly translated into empirical formulas used to construct treatment units that commonly failed (Peters and Foley, 1983).

One of the roles of an engineer is to make predictions using scientific principals. Often the primary tool of the engineer is a mathematical model. A mathematical model is a numerical algorithm that can be used to translate input variables into output variables and can generally be described as empirical or mechanistic. Empirical models typically describe a simple relationship between input and output variables based on the mathematical analysis of trends from research data and do not attempt to describe the underlying phenomena. These "black-box" models are often used to design systems based on pilot plant data from complex or poorly understood processes. Mechanistic models, on the other hand, are derived from mass balances performed on a system's essential components and describe the underlying phenomena in such a way that performance may be predicted outside the range of tested conditions (Grady, 1983).

A common problem in the engineering of biological systems is that new systems designed from empirical models often fail. The common cause of these failures is that the fundamental requirements of biological systems are often so poorly understood that new systems are designed lacking the essential requirements necessary for system success.

In order to quantitatively describe the influence of nitrifying organisms in aquatic systems it is necessary to know their growth rates, substrate utilization rates and the relationships between these rates and the system conditions with which they interact. The study of the rates at which these reactions take place is termed kinetics (Grady, et al., 1999).

Through the analysis of experimental data, Monod (mo nô´) (1949) recognized that bacterial growth in a limiting substrate could be represented by the following empirical hyperbolic formula relating the bacterial specific growth rate to substrate concentration.

$$\mu = \hat{\mu}\left[\frac{S}{K+S}\right] \qquad (2.4\text{-}1)$$

where

| | | | |
|---|---|---|---|
| $\mu$ | = | specific growth rate | ($hr^{-1}$) |
| $\hat{\mu}$ | = | max specific growth rate | ($hr^{-1}$) |
| S | = | substrate concentration | (mg/L) |
| K | = | half-saturation constant | (mg/L) |

Selecting typical kinetic parameters from Grady and Lim (1980) of:

| | | |
|---|---|---|
| $\hat{\mu}$ | = | 0.032 $hr^{-1}$ |
| $K_{NH}$ | = | 1.0 mg/L N |

we can plot the model over ranges encountered in aquaculture (Figure 2.4-1).

The applicability of the Monod equation has been substantiated through so many observations that it is today considered to be a basic concept of microbial kinetics. Though the Monod equation is empirical, it is essentially identical to the mechanistic Michaelis-Menten equation, which can be derived from the rates of chemical reactions catalyzed by enzymes (Knowles, et al., 1965; Halling-Sorensen and Jorgensen, 1993; Grady, et al., 1999). Thus, the Michaelis-Menten equation can be viewed as a mechanistic validation of the empirical Monod equation, and some scientists present

their work in Michaelis-Menten terms rather than those of the Monod equation (Knowles, et al., 1965).

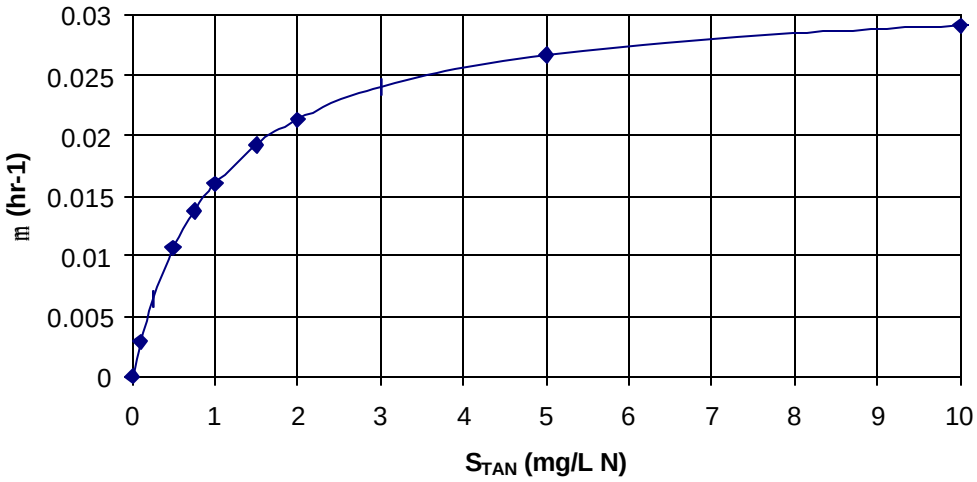


Figure 2.4-1. Monod model for typical aquaculture conditions.

When micronutrient and $O_2$ requirements are met, the limiting substrate for *Nitrosomonas* is $NH_4^+$ and for *Nitrobacter* is $NO_2^-$. The growth rate for *Nitrobacter* is higher than that of *Nitrosomonas*, thus the oxidation of $NH_4^+$ is usually the controlling reaction of the two stages of nitrification (Water Pollution Control Federation, 1983; Halling-Sorensen and Jorgensen, 1993). Therefore most literature, when reporting the kinetic parameters or conversion rates of nitrification, present nitrification rates in terms of only the oxidation of $NH_4^+$.

When a microorganism must rely on two or more potentially growth limiting substrates, a multi-substrate model is employed. Equation 2.4-2 shows the interactive Monod model for $O_2$ and $NH_4^+$, the most widely used for substrate limited nitrification kinetics (Grady and Lim, 1980).

$$\mu = \hat{\mu}\left[\frac{S_O}{K_O + S_O}\right]\cdot\left[\frac{S_{NH}}{K_{NH} + S_{NH}}\right] \qquad (2.4\text{-}2)$$

where

| | | | |
|---|---|---|---|
| $\mu$ | = | specific growth rate | ($hr^{-1}$) |
| $\hat{\mu}$ | = | max specific growth rate | ($hr^{-1}$) |
| $S_O$ | = | $O_2$ concentration | (mg/L $O_2$) |
| $K_O$ | = | $O_2$ half-saturation constant | (mg/L $O_2$) |
| $S_{NH}$ | = | $NH_4^+$-N concentration | (mg/L N) |
| $K_{NH}$ | = | $NH_4^+$-N half-saturation constant | (mg/L N) |

Selecting typical kinetic parameters from Grady and Lim (1980) of:

| | | |
|---|---|---|
| $\hat{\mu}$ | = | 0.032 $hr^{-1}$ |
| $K_O$ | = | 0.75 mg/L $O_2$ |
| $K_{NH}$ | = | 1.0 mg/L N |

we can plot the model over ranges encountered in aquaculture (Figure 2.4-2).

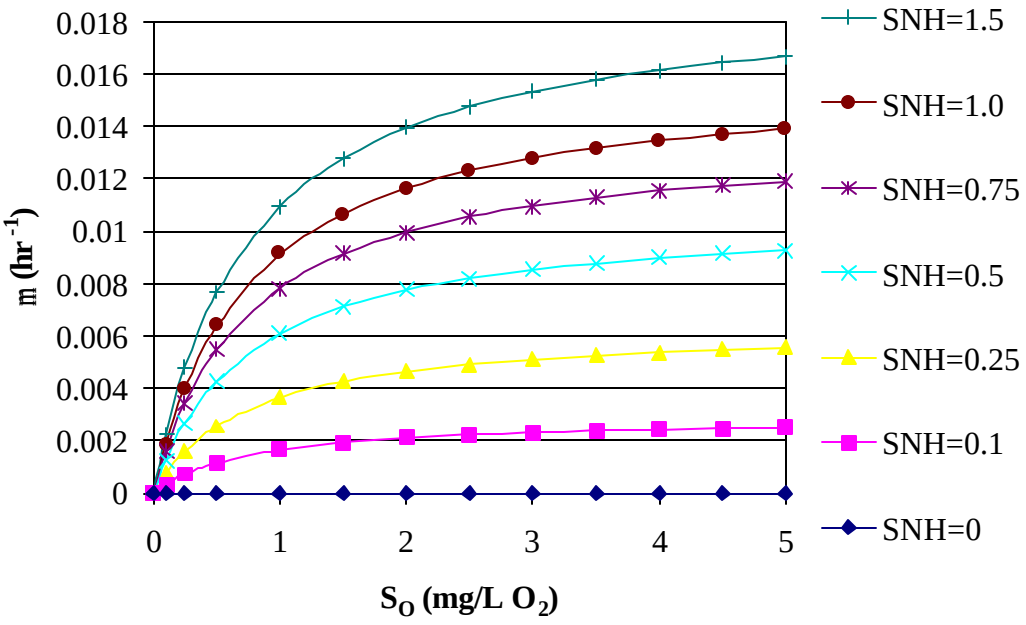


Figure 2.4-2. Interactive Monod model for typical aquaculture conditions. Note: SNH depicts $NH_4^+$-N (TAN equivalent) concentrations in mg/L.

From Figure 2.4-2 we can observe the region where TAN becomes the limiting factor where the slopes change from linear to curved; however this model depicts

suspended-growth cultures and may not apply in certain fixed-film applications where diffusion plays a major role (Zang et al., 1995).

## 2.5 Factors Affecting Nitrification

Several critical factors impacting nitrification through complex interactions are identified throughout the literature.

### 2.5.1 Dissolved Oxygen

Though the interactive Monod model for $O_2$ and TAN (as $NH_4^+$-N) was introduced in Section 2.4 as the kinetic model most widely used for substrate limited nitrification kinetics, this model depicts suspended-growth cultures and may not best represent fixed-film applications where diffusion plays a major role (Zang et al., 1995).

In fixed-films, nutrients must diffuse from the bulk liquid through a laminar layer and finally through the biofilm itself to reach the bacterial cells. Since different substrates have different diffusion rates through these layers, selection of the limiting substrate becomes more involved than previously mentioned. Several authors have proposed simple equations based on stoichiometry and diffusion to predict the limiting substrate for nitrifying biofilms. One such model presented by Zang et al. (1995) is given below (Equation 2.5.1-1).

$$\frac{S_N}{S_O} = \frac{\upsilon_N}{\upsilon_O}\frac{D_O MW_N}{D_N MW_O} \qquad (2.5.1\text{-}1)$$

where

| | | | |
|---|---|---|---|
| $S_N$ | = | bulk concentration $NH_4^+$ | (mg/L $NH_4^+$) |
| $S_O$ | = | bulk concentration $O_2$ | (mg/L $O_2$) |
| $\upsilon_N$ | = | stoichiometric constant for $NH_4^+$ | |
| $\upsilon_O$ | = | stoichiometric constant for $O_2$ | |
| $D_O$ | = | biofilm diffusivity $O_2$ | ($m^2$/day) |
| $D_N$ | = | biofilm diffusivity $NH_4^+$ | ($m^2$/day) |
| $MW_N$ | = | molecular weight $NH_4^+$ | |
| $MW_O$ | = | molecular weight $O_2$ | |

From equation 2.3-3 we know that $\upsilon_N$ and $\upsilon_{DO}$ are equal to 1 and 2, respectively. If we let $D_N$ and $D_{DO}$ equal $1.7x10^{-4}$ and $2.2x10^{-4}$ $m^2/d$ (Zang et al., 1995), then the ratio $S_N/S_{DO}$ in Equation 2.5.1-1 equals 0.365. Therefore, if the TAN concentration is 1 mg/L (1 mg/L TAN = 1.29 mg/L $NH_4^+$) then any $O_2$ concentration less than 3.54 mg/L will cause $O_2$ to be the rate limiting substrate.

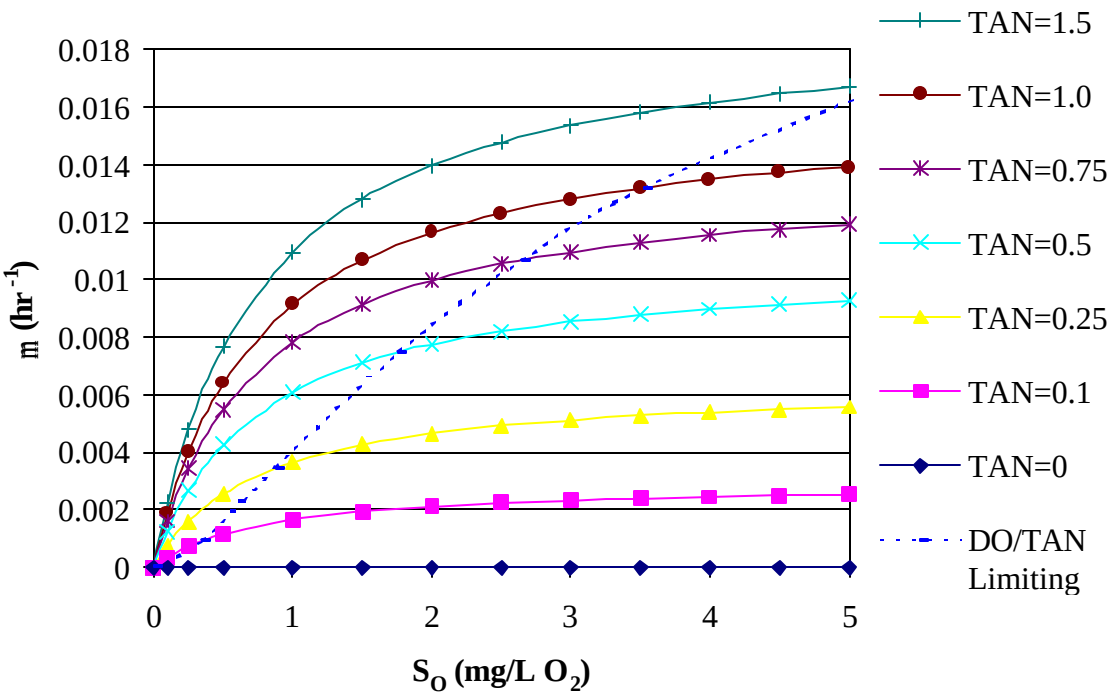


Figure 2.5.1-1. DO to TAN limiting boundary (Equation 2.5.1-1) applied to the interactive Monod model for typical aquaculture conditions (Figure 2.4-1).

Solving Equation 2.5.1-1 for $S_N$ and plugging the result into Equation 2.4-2 allows a line to be plotted across Figure 2.5.1-1 showing the boundary between $O_2$ limiting (left of dashed line) and TAN limiting (right of dashed line) conditions.

Of course, Equation 2.5.1-1 is a simplification of the processes at work in actual systems and may not accurately describe stratified biofilms or biofilms in which the nitrifiers and heterotrophs are not uniformly distributed (Zang et al., 1995).

### 2.5.2 Temperature

There is considerable variation in the literature on the effects of temperature on nitrifier growth rate or activity (Painter, 1970; USEPA, 1975; Randall and Buth, 1984; Hochheimer, 1990; Wheaton et al., 1991; Mia, 1996; Grady et al., 1999). Randall and Buth (1984) suggest that these reported differences are likely due to the influence of other critical parameters and Wheaton et al. (1991) suggests that nitrifiers will adapt to a variety of temperatures provided they are given time to do so. However, more recent research indicates that several different organisms may be responsible for nitrification, with different species dominating as conditions, substrates, or locations change (Watson et al., 1981; Wagner et al., 1996; Jones et al., 2000).

It is generally accepted that between two critical temperatures, nitrification rates increase with increasing temperature (Sharma and Ahlert, 1977; Thomasson, 1991) and that the effect of temperature on the maximum specific growth rate ($\hat{\mu}$) fits an Arrhenius-type equation over this range (Grady, 1980). The change in nitrifier activity over this temperature range has been reported as substantial (Grady, 1980), however it has also been shown that for certain fixed-film systems the response to temperature may be minor over specific ranges (Forgie, 1984). This raises the possibility that fixed-film systems may be less sensitive to changes in temperature than other systems, especially over the lower range (USEPA, 1975; Drapcho, 1986; Halling-Sorensen and Jorgensen, 1993; Iwai and Kitao, 1994). Grady (1980) states that as a general rule the published equations should be used only to approximate the effects of temperature and that the actual effects should be determined using treatability studies.

The temperature range of 28 to 36 °C has been commonly cited (Thomasson, 1991; DeLosReyes, 1995; Sastry, 1996) as an optimum growth range for nitrifiers based on a summary by Sharma and Ahlert (1977) of the Painter (1970) literature review. In defining an optimal temperature range it is important to consider the physiology of both nitrifier groups as nitritation and nitratation activities can deactivate or be severely retarded at different critical temperatures (Grady, 1980). Therefore, the substrate utilization of one nitrifier may be negatively impacted when its critical temperature is reached before the optimal temperature is reached for the other nitrifier, resulting in the accumulation of either TAN or $NO_2^-$-N in recirculating system. Wortman and Wheaton (1991) demonstrated that for a particular fixed-film system, nitrification was linearly related to temperature in the range from 7 to 35 °C. But due to $NO_2^-$-N accumulation at higher temperatures, they recommended 25 °C as optimum for similar systems and conditions.

2.5.3 pH

Numerous studies have been conducted to determine the effects of pH on nitrification (Lawson, 1995; Sastry, 1996, Flora et al., 1999a) and these studies promote the following four primary concepts that are not mutually exclusive as pH is both effected by and in turn affects several complex solubility and equilibria reactions.

1. Nitrification can be strongly pH dependant (Wild et al., 1971; Siegrist and Gujer, 1987, Flora et al., 1999a).
2. Nitrification system pH-dependence can be temporary (<15 days) for systems operated within a specific pH range (Wheaton et al., 1994), such as 6 to 9 and

which may depend on community shifts and other complex factors (Siegrist and Gujer, 1987; Prinèiè et al., 1998).

3. The effect of low pH on nitrifiers may only be substantial when buffer capacity is inadequate (Flora et al., 1999a) or alkalinity is below 75 mg/L as $CaCO_3$ (Siegrist and Gujer, 1987).
4. The pH-dependence of nitrifiers show a sensitivity to biofilm thickness, partially because pH decreases across the biofilm due to nitrification (Flora et al., 1999b).

The exact mechanism by which pH affects nitrification is unclear, however there have been numerous published observations and hypotheses. Siegrist and Gujer (1987) observed over short-term periods (<2 days) that as pH drops, $NH_4^+$-N oxidation decreases as a result of a decrease in the maximum volumetric uptake rate and an increase in the Monod half saturation constant ($K_{NH}$) for $NH_4^+$-N. Concurrently, an increase in $NO_2^-$ results as the $NO_2^-$ Monod half saturation constant ($K_{NO}$) decreases. Therefore, as pH drops the steady state concentration of $NO_2^-$ is significantly reduced. Alternatively, Sharma and Ahlert (1977) and Prinèiè et. al (1998) presented the Anthonisen (1974) hypothesis that the mechanism is related to $NH_3$ $NH_4^+$ (see Section 2.2.1.3) and $NO_2^-$ $HNO_2$ pH dependant equilibria. More recently, Flora et al. (1999a) suggested that ionic interactions, chemical equilibrium, and electroneutrality should be considered. Another suggestion was offered by Painter (1970) who stated that errors in published experiments could be introduced if either the solutions used were not buffered against acid produced during nitrification (see Equation 2.3-1), or if buffers were used that had anions producing inhibitory effects.

Painter (1970) presented the findings of Winogradsky and Winogradsky (1933) as what remains to be perhaps the widest variation reported for the optimal growth of *Nitrosomonas* (pH ranging from 6 to 9 for 6 strains) and *Nitrobacter* (pH ranging from 6.3 to 9.4 for 7 strains). Though the pH optima are not sharply defined, they are normally on the alkaline side of neutrality (>7.0) (Painter, 1970; Sharma and Ahlert, 1977; Lawson, 1995). For nitrification in fresh (< 10 ppt salinity) warmwater recirculating aquaculture systems, a pH in the range of 7.5 to 8.0 has been recommended for optimal nitrification while remaining low enough to minimize unionized $NH_3$ (Thomasson, 1991; Coffin, 1993; Loyless and Malone, 1997).

2.5.4 Alkalinity

In addition to the buffering effects on pH mentioned in the previous section, the conversion of $NH_4^+$ to $NO_3^-$ consumes alkalinity at the rate of 8.64 mg $HCO_3^-$ (14.7 mg as $CaCO_3$) per mg of $NH_4^+$-N oxidized to $NO_3^-$ (see Equation 2.3-6). Paz (1984) demonstrated that nitrification efficiency was affected more by variations in alkalinity than by pH and also recommended a minimum concentration of 100 mg/L (Mia, 1996; Sastry, 1996). Malone and Burden (1988a, 1988b) showed that bicarbonate ($HCO_3^-$) alkalinity may be critical for the growth of nitrifying organisms and noted that nitrification was inhibited when system alkalinity fell below 100 mg/L as $CaCO_3$.

It should be noted that a pH dependant equilibria exists between carbon dioxide ($CO_2$) and alkalinity. In order to maintain $CO_2$ concentrations below the animal stress level of 15 mg/L and to prevent a potential ion imbalance, Loyless and Malone (1997) recommend a maximum alkalinity concentration of 200 mg/L as $CaCo_3$ for nitrification

in fresh (< 10 ppt salinity) warmwater recirculating aquaculture systems for the pH range 7.5 to 8.0.

2.5.5 Nitrogen

The most familiar mechanisms by which inorganic nitrogen composition and concentration affect nitrification involve growth and substrate utilization and include:

1. the direct impact of substrate concentration on the nitrifying organism's specific growth rate (see Sections 2.4 and 2.5.1);
2. immediate changes in system performance if substrate concentration changes cause the limiting substance to switch from one to another (i.e., $NH_3$ to $O_2$); and
3. long-term changes in system performance from changes in substrate concentration, which may take days or weeks to observe as nitrifiers have low growth rates and are slow to respond (Boller et al., 1997).

Other mechanisms involve inhibition and include:

1. nitrification inhibition by both $NH_3$ and $HNO_2$ (function of pH - see Section 2.5.3);
2. inhibition of *Nitrosomonas* and *Nitrobacter* by the substrate of each other at concentrations above approximately 500 mg N /L(Painter, 1970); and
3. inhibition of *Nitrosomonas* and *Nitrobacter* by their own substrate at concentrations above approximately 1,400 mg N /L(Painter, 1970).

2.5.6 Biomass

In order to describe quantitatively the influence of nitrifying organisms in aquatic systems it is necessary to know their growth constants under relevant environmental

conditions, and the half saturation constants of the substrates which support their growth. It is also desirable to be able to estimate the concentration of these organisms present in a given sample so that their effect can be evaluated against substrate utilization rates.

To demonstrate the effect of biomass on substrate utilization rates we can use the simple kinetic equations for a single limiting substrate in a batch reactor. The equations were demonstrated by Knowles et al. (1965) to conform fairly closely to the growth of both *Nitrosomonas* and *Nitrobacter*. These equations can also later be used to evaluate startup acclimation performance, where the experimental systems are operated as batch reactors with inorganic N as the single limiting substrate and initial biomass concentrations may play an important role.

$$\frac{dx_{NS}}{dt} = \mu_{NS} x_{NS} \qquad (2.5.6\text{-}1)$$

where

| | | | |
|---|---|---|---|
| $x_{NS}$ | = | *Nitrosomonas* concentration as dry matter | (mg dry cells/L) |
| $\mu_{NS}$ | = | *Nitrosomonas* specific growth-rate | ($day^{-1}$) |
| t | = | time | (days) |

or substituting Equation 2.4-1

$$\frac{dx_{NS}}{dt} = \frac{\hat{\mu}_{NS} S_{NH} x_{NS}}{S_{NH} + K_{NH}} \qquad (2.5.6\text{-}2)$$

where

| | | | |
|---|---|---|---|
| $\hat{\mu}_{NS}$ | = | *Nitrosomonas* maximum specific growth-rate | ($day^{-1}$) |
| $S_{NH}$ | = | ammonia concentration | (mg TAN/L) |
| $K_{NH}$ | = | ammonia half saturation or Michaelis constant | (mg TAN/L) |

The corresponding equation for the growth of *Nitrobacter* is

$$\frac{dx_{NB}}{dt} = \frac{\hat{\mu}_{NB} S_{NO2} x_{NB}}{S_{NO2} + K_{NO2}} \qquad (2.5.6\text{-}3)$$

where

| | | | |
|---|---|---|---|
| $x_{NB}$ | = | *Nitrobacter* concentration as dry matter | (mg dry cells/L) |
| $\hat{\mu}_{NB}$ | = | *Nitrobacter* growth-rate constant | ($day^{-1}$) |
| $S_{NO2}$ | = | nitrite concentration | (mg $NO_2^-$-N/L) |
| $K_{NO2}$ | = | nitrite half saturation or Michaelis constant | (mg $NO_2^-$-N/L) |
| t | = | time | (days) |

If the oxidation of unit mass of ammonia-N produces a dry mass $E_m$ of *Nitrosomonas* organisms then

$$x_{NS} - x_{NSo} = Y_{NS}(S_{NHo} - S_{NH}) \qquad (2.5.6\text{-}4)$$

where

| | | | |
|---|---|---|---|
| $x_{NSo}$ | = | initial *Nitrosomonas* concentration as dry matter | (mg dry cells/L) |
| $Y_{NS}$ | = | Yield for *Nitrosomonas* | [(mg dry cells/L)/(mg TAN/L)] |
| $S_{NHo}$ | = | initial ammonia concentration | (mg TAN/L) |

Combining Equation 2.5.6-4 and Equation 2.5.6-2 gives a differential equation that can be integrated to a form that gives the relation between TAN concentration and time:

$$\hat{\mu}_{NB} t = \left(\frac{1}{A}\right)\left[Y_{NS}K_{NH} \cdot \ln\left(\frac{S_{NHo}}{S_{NH}}\right) + (A + Y_{NS}K_{NH})\ln\left(\frac{A - Y_{NS}K_{NH}}{x_{NSo}}\right)\right] \qquad (2.5.6\text{-}5)$$

where

$$A = x_{NSo} + Y_{NS}S_{NHo} \qquad (2.5.6\text{-}6)$$

Recognizing that Equation 2.5.6-4 may also be represented as:

$$x_{NS} - x_{NSo} = -Y_{NS}(S_{NH} - S_{NHo}) \qquad (2.5.6\text{-}7)$$

or

$$\frac{dx_{NS}}{dS_{NH}} = -Y_{NS} \tag{2.5.6-8}$$

$$dx_{NS} = -Y_{NS} dS_{NH} \tag{2.5.6-9}$$

$$x_{NS} = Y_{NS}(S_{NHo} - S_{NH}) + x_{NSo} \tag{2.5.6-10}$$

We may now substitute Equation 2.5.6-9 and Equation 2.5.6-10 into Equation 2.5.6-2, which becomes:

$$\frac{dS_{NH}}{dt} = -\frac{\hat{\mu}_{NS}(Y_{NS}(S_{NHo} - S_{NH}) + x_{NSo})S_{NH}}{Y_{NS}(S_{NH} + K_{NH})} \tag{2.5.6-11}$$

In extending the calculation to the changes in concentration of $NO_2^-$-N with time the concentration of *Nitrobacter* is given by an equation similar to Equation 2.5.6-4:

$$x_{NB} - x_{NBo} = Y_{NB}\{S_{NO2o} + f_m(S_{NHo} - S_{NH}) - S_{NO2}\} \tag{2.5.6-12}$$

where

| | | | |
|---|---|---|---|
| $x_{NBo}$ | = | initial *Nitrobacter* concentration as dry matter | (mg dry cells/L) |
| $Y_{NB}$ | = | Yield for *Nitrobacter* | [(mg dry cells/L)/(mg $NO_2^-$-N/L)] |
| $S_{NO2o}$ | = | initial nitrite concentration | (mg $NO_2^-$-N/L) |
| $f_m$ | = | ammonia to nitrite conversion ratio accounting for *Nitrosomonas* biomass formation | [(mg $NO_2^-$-N/L)/ (mg TAN/L)] |

As with Equation 2.5.6-4 we will rearrange Equation 2.5.6-12 into the two necessary forms:

$$dx_{NB} = -Y_{NB} dS_{NO2} \tag{2.5.6-13}$$

$$x_{NB} = Y_{NB}(S_{NO2o} + f_m(S_{NHo} - S_{NH}) - S_{NO2}) + x_{NBo} \tag{2.5.6-14}$$

Substituting Equations 2.5.6-13 and Equation 2.5.6-14 into Equation 2.5.6-3 yields:

$$\frac{dS_{NO2}}{dt} = -\frac{\hat{\mu}_{NB}\left[Y_{NB}\left(S_{NO2o} + f_m\left(S_{NHo} - S_{NH}\right) - S_{NO2}\right) + x_{NBo}\right]S_{NO2}}{Y_{NB}\left(S_{NO2} + K_{NO2}\right)} \qquad (2.5.6\text{-}15)$$

Therefore, as can be seen in Equations 2.5.6-11 and 2.5.6-15 the rate of substrate utilization is directly proportional to biomass concentration provided that other parameters are not limiting.

2.5.7 Organic Loading

With the exception of pure cultures grown using sterile techniques, it has been shown that while heterotrophs may exist without nitrifiers, the reverse is not so. For cultures growing on pure $NH_4^+/NH_3$, heterotrophs supported by soluble autotrophic products and cell debris can populate the biofilm in the same order of magnitude as the target autographs (Zang et al., 1995). These heterotrophs not only place an additional $O_2$ demand on the biofilm but may also increase TAN concentrations (Hanaki et al., 1990), especially in aquacultural or waste-waters containing organic nitrogen, through the process known as heterotrophic ammonification or mineralization (Bovendeur et al., 1990). This increased $O_2$ demand may inhibit nitrification by reducing available $O_2$ in thick diffusion-limited biofilms, more so than in thin biofilm systems. In addition to substrate factors, heterotrophs are capable of out-competing autotrophic nitrifiers for physical space (Hanaki et al., 1990) as they typically have maximum growth rates five times and yields two to three times greater than those of nitrifiers (Grady and Lim, 1980). The effects of organic mater on nitrification was demonstrated by Okabe et al. (1996) who found that nitrification was inhibited at C:N ratios of 1.5 and higher while a ratio of 0.25 produced the fastest accumulation of nitrifiers relative to heterotrophs. Similarly, Grady and Lim (1980) observed maximum nitrification rates at a $BOD_5$/TKN ratio of 0.25 with decreasing nitrification as the ratio increased.

In aquaculture systems the concentration of organic matter is typically high compared to the requirement for low TAN concentrations. Yet, even though the effects of organic matter on nitrification are well established, quantitative data regarding these effects are scarce (Zhu and Chen, 2001b). Few aquaculture researchers record data related to organic content other than total suspended solids (TSS), which can be affected by inert fish feed fillers and bulking agents as well as algae.

## 2.6 Attached Growth Reactors

A fixed-film or "biofilm" process utilizes attached or immobilized microbes to achieve the desired results. The three broad categories of microbial immobilization methods include the carrier binding method, entrapment, and the cross-linking method with the biofilm process considered as a type of carrier binding method (Iwai and Kitao, 1994).

Although the term "fixed-film" in itself describes a static film, processes where the biofilm is grown on solid inert media can be divided into either static or dynamic types based on media action. In water treatment applications, static media systems include submerged biological filters, trickling filters, and expandable granular media "bead" filters, while dynamic media systems include fluidized bed (including sand) filters, spouted-bed filters (including the CCMB), and rotating biological contactors (RBCs). Conversely, activated sludge systems are systems without media where the bacteria form dynamic slurries of biofilm particulates or floc.

### 2.6.1 Cell Retention

All suspended growth bioreactors/biofilters are designed with and must be operated at a dilution rate $D < Dc$, where Dc is the critical dilution rate at which cells can complete at least one cell division before being washed from the culture. This condition

allows cells to remain for a sufficient length of time in the bioreactor in order to perform the desired function (Biotol, 1992).

On the other hand, at dilution rates above the wash-out rate (D > Dc), only attached cells remain in the bioreactor as the suspended cells are washed out. Therefore, fixed-film bioreactors can be operated above the critical dilution rate (Biotol, 1992).

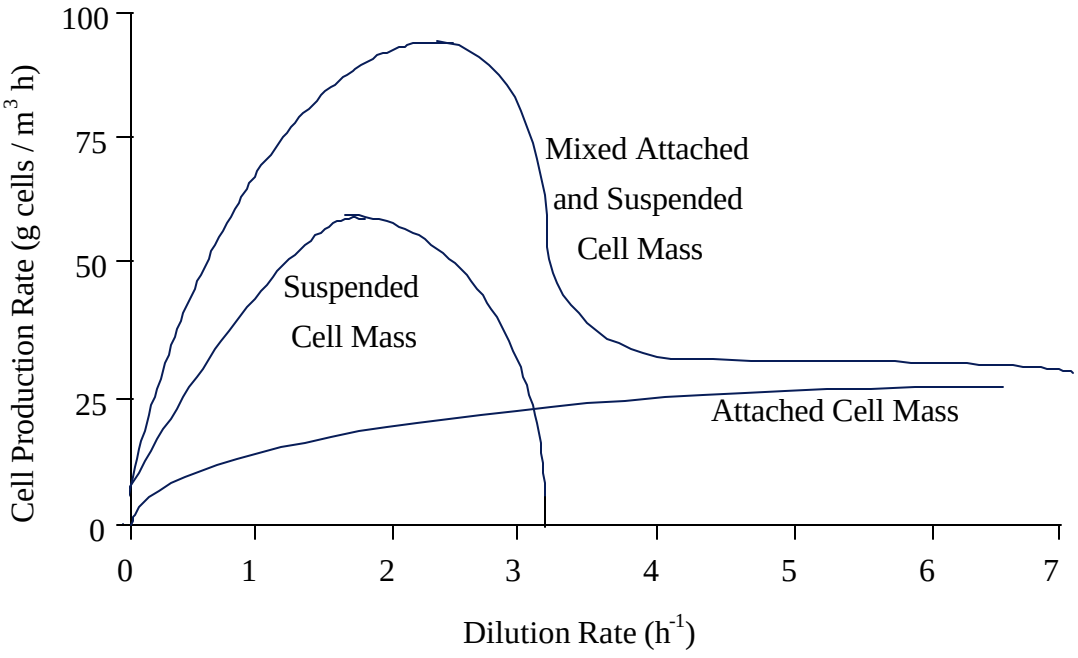


Figure 2.6.1-3. Typical models showing cell mass production rate against dilution rate (rendered after Biotol, 1992).

This can be illustrated by comparing the cell mass productivity of attached and suspended growth systems (Figure 2.6.1-3). The rate of cell mass production (proportional to the rate of substrate consumption) in a suspended growth bioreactor is determined by the duration of the cells in the bioreactor and by the availability of substrate. For the suspended growth case, a plot of the rate of substrate consumption versus dilution rate shows a gradual rise in cell mass production rate until a maximum is reached, followed by a drop to zero at the critical dilution rate (Biotol, 1992).

This gradual rise due to increased substrate availability with increasing dilution rate also occurs with attached growth systems, however once the point at which the availability of substrate is maximized, the cell mass production rate approaches steady-state in spite of an increase in dilution rate. This curve closely resembles that of a saturation model (Biotol, 1992).

A mixed attached and suspended growth system productivity versus dilution rate plot is also shown in Figure 2.6.1-3. It should be noted that for a mixed process there is never a critical decrease in biomass productivity (Biotol, 1992).

2.6.2 Biofilm

Besides cell retention, the most dramatic difference between suspended growth and fixed-film cultures is the potential for diffusion limitations resulting from the biofilm structure. Therefore, mechanistic, diffusion-based, models are often used to describe biofilm systems (Grady, 1983; Atasi and Borchardt, 1984) and it is important to understand the biofilm structure so that it can be reduced down to its essential components (Grady, 1983).

The development of a typical biofilm on the surface of a clean solid surface or media can be divided into three stages, although not all stages may be observed in all systems. In the first stage, the biofilm is thin and all microorganisms are in an exponential growth phase. The entire thickness of the biofilm is active and is not considered diffusion limited because either the biofilm is thin enough, or the diffusion coefficient of the compound(s) low enough that the substrate utilization rate is limited by reaction kinetics and not diffusion. Next, the biofilm grows to a point at which its thickness is greater than the layer of microorganisms producing the desired effect

(effective depth). The effective depth is predominantly a function of environmental factors such as the diffusion of the electron acceptor and donor through both the liquid film and biofilm. Therefore it remains steady at this stage of development. With the total amount of growing microorganisms constant during this stage the metabolism switches to maintenance mode. At the third stage, the thickness of the biofilm reaches a plateau, where the growth rate is fully balanced with its environment via biomass loss and diffusion. Unfortunately, the effective depth of the biofilm generally decreases at this stage. Some systems never reach a discrete plateau, continuing to grow until either, the layers closest to the solid substrate become diffusion limited enough that large portions of the biofilm slough off, or the system becomes overgrown and clogs/biofouls (Characklis, 1981; Iwai and Kitao, 1994).

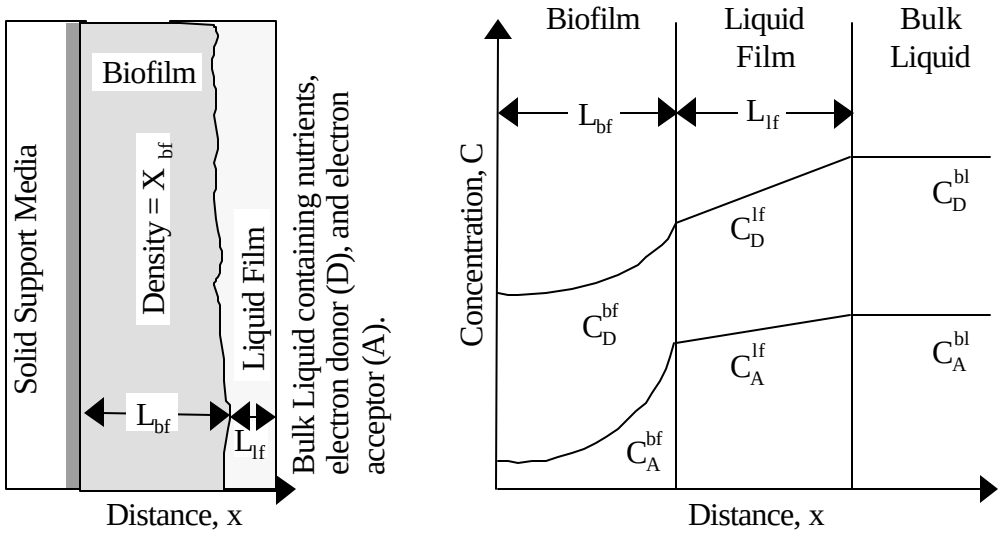


Figure 2.6.2-1. Simplified biofilm structure and concentration profiles (adapted from Grady, 1983; Atasi and Borchardt, 1984; and Sáez, et al., 1984).

2.6.3 Turbulent Diffusion & Shear

It is difficult to discuss turbulent diffusion without also discussing shear since both are products of fluid flow and can significantly affect fixed-film systems.

Fluid flow can be either laminar or turbulent depending upon the ratio of inertia forces to viscous forces within the fluid as expressed by the nondimensional Reynolds Number (RN). Flows are considered laminar where the RN < 2000, turbulent where the RN > 4000, and in transition where 2000 < RN < 4000 (Lawson, 1995).

The most obvious effect of turbulence, or lack thereof, is its effect on the thickness of the liquid film layer ($L_{lf}$ in Figure 2.6.2-1), sometimes referred to as the laminar film layer (Iwai and Kitao, 1994), stagnant film layer (Hochheimer, 1990; Wheaton et al., 1994a) or static film layer. The rate of nutrient diffusion through the liquid film layer is inversely proportional to its $L_{lf}$ according to Fick's law of diffusion (Grady et al., 1999), with a higher RN resulting in a lower $L_{lf}$ down to some critical or minimum $L_{lf}$ value (Williamson and McCarty, 1976).

Turbulent diffusion, as the term implies, refers to the condition where, at higher RNs, diffusion limitations from the liquid film are reduced as the $L_{lf}$ is reduced and turbulence induced "downsweeps" carry material from the bulk fluid directly to the biofilm (Characklis, 1981). With optimal turbulent diffusion, the transport rate of reactants to the biofilm exceeds the maximum reaction rate (Grady, 1983). Therefore, turbulent diffusion can have a significant impact on the performance of a biofilm system (Tanaka and Dunn, 1982).

Zhu and Chen (2001a) showed that for a nitrifying biofilm growing on the inner walls of flexible tubing, raising the RN above 4000 had a significant impact on TAN removal while there was no significant increase in the maximum TAN removal rates between RNs of approximately 2000 and 4000. They also observed that for a fixed TAN concentration, the TAN removal rate at RN = 66,710 was approximately 5 times

greater that at RN = 1,668. During these trials, biofilm shear was only observed when the RN was increased rapidly from low to high. This proved to be only a temporary condition, with the biofilm acclimating to the new flow conditions and becoming reestablished after several days. Similarly, Mia (1996) reported increased nitrification rates and conversion efficiencies as a result of increased turbulence on nitrifying biofilms grown in hoses, and other past studies on heterotrophic biofilms achieved maximum growth through turbulent diffusion at higher fluid velocities (Sanders, 1966; Characklis, 1967).

Other effects of increased turbulence include the observations that higher velocities retard initial biofilm formation, but once established, increasing RN results in greater growth (Heukelekian, 1956a); and biofilms grown at higher velocities adhere to surfaces more firmly (Characklis, 1967).

Shear describes a mechanism beyond turbulent diffusion where at higher RN values, the $L_{lf}$ is reduced down far enough towards the media that some of the biofilm is sheared or scoured off, resulting in a lower $L_{bf}$. Like in the case of turbulent diffusion, there is a critical lower thickness at which the diffusion rate exceeds the maximum reaction rate. There has been supposition, but no solid experimental evidence of a direct correlation between the critical thickness and the effective depth (Zhang et al., 1995).

Obviously turbulence is capable of thinning the biofilm by increasing shear, which could either reverse the stages of biofilm development described in the previous subsection, or maintain a biofilm at a lower stage continuously. Recalling that Stage 1 biofilms are in a state of exponential growth and Stage 2 biofilms have the maximum

effective layer, turbulence is considered an excellent tool for improving process efficiency by controlling biofilm activity through thin-film operations (Burden, 1988; Liu and Capdeville, 1996).

Besides thinning the biofilm, it has been suggested that the turbulence induced shear effect of higher nitrification rates may also be due to turbulence disrupting the biofilm and creating a larger effective surface area for the nitrifiers (Eighmy and Bishop, 1984). However, turbulence can increase substrate removal rates only so far, after which further increases in turbulence and the resulting shear forces produce too much nitrifier biofilm removal (too thin a film) and lowered substrate removal rates (Eighmy and Bishop, 1984; Burden, 1988; Chang et al., 1991; Liu and Capdeville, 1996).

Finding the ideal biofilm thickness is difficult to do other than experimentally, because of the complex interactions between interrelated conditions as illustrated by the following observations.

- Substrate concentration affects biofilm thickness and density (Chang et al., 1991).
- Increasing nutrient concentration increases the biofilm thickness (Sandu et al., 2002) and detachment rate (Chang et al., 1991).
- Increasing turbulence can produce denser (Chang et al., 1991) and thinner biofilms (Chang et al., 1991; Sandu et al., 2002).
- Denser and thinner biofilms are less sensitive to both shear and abrasion biofilm losses (Nicolella, et al., 1997), while the opposite was observed by Nam et al. (2000).

- Biofilm structure affects substrate transfer and competition (Zhang et al., 1995).
- Attachment characteristics change depending on the species or the physiological condition of the microorganism (Characklis, 1973).
- Species distribution varies with turbulence (Characklis, 1971).
- Attachment rate is lower and detachment rate is higher for nitrifiers than for heterotrophs (Oga, et al., 1991).

2.6.4 Conventional Attached Growth Reactor Configurations

So many biofilter configurations have been used in semi-closed fish systems that it is impractical to list them all. This is partly due to the fact that many individuals are encouraged to construct their own biofilters, perhaps because of the same innovative tendencies which led them to the industry (Lutz, 1996). However, submerged, trickling, rotating biological contactor (RBC), and fluidized bed sand biofilters are the most common configurations utilized by the aquaculture industry (Lawson, 1995; Wheaton et al., 1991).

Of these configurations fluidized bed sand filters have consistently proven to be among if not the best for performing biofiltration in aquaculture (Westerman et al., 1993; Thomasson, 1991; Owsley et al., 1989; Burden, 1988; Miller and Libey, 1985; Nam et al., 2000), wastewater (Tang and Fan, 1987; Fan et al., 1987), and solid waste land fill leachate systems (Martienssen et al., 1995).

Despite the apparent benefits of non-clogging, high surface area to volume ratio, high nitrification rates, high biomass concentrations, high hydraulic loading rates, and intimate contact between the liquid and solid (sand/biofilm) phase (Tang and Fan,

1987), the fluidized bed sand filter is not without disadvantages. Among these are the control of bed expansion below wash-out or bed carry-over (Chang et al., 1991) and required high flow rates over the entire cross-section for fluidization (Wheaton et al., 1991).

**2.7 Significance of Filter Media Characteristics**

It seems that one can rarely open a wastewater, aquaculture, or even aquarium magazine or journal article without seeing an article describing some novel biofilter media. There is perhaps an unlimited list of potential media as practically any solid (phase) material that can be contained in a system and is non-toxic to the biofilm, the culture crop, and the environment can be utilized (Wheaton et al., 1994; Lawson, 1995). Media ranging in size from fine-grained sand (Wheaton et al., 1994; Lawson, 1995) to large concrete blocks or rip-rap (Kadlec & Knight, 1996) and materials ranging from inorganic media such as silica and carbonate-based rock and shell to plastics (Wheaton et al., 1994; Lawson, 1995), and even biological materials such as living plant roots (Rakocy and Hargreaves, 1993), wooden pallets (Lawson, 1995) and compost (Caissel, 1991) have been used.

The two most important physical characteristics of any nitrification system biofilter media are a high surface area to volume ratio or specific surface area, and low clogging (biofouling) properties (Wheaton et al., 1994; Lekang and Kleppe, 2000). The latter is often strongly influenced by the hydraulic characteristics of the particular biofilter as what may clog in one system may thrive in another. Other characteristics for consideration include durability, availability, cost, and the often related characteristics of specific gravity, weight, and buoyancy (Lawson, 1995).

For biofilters with higher turbulence or shear, surface roughness may be an important media physical characteristic. Observations have been reported that increased surface roughness can significantly decrease the initial biofilm-forming period and that the attached amount on a rough surface is larger than that on a smooth surface (Heukelekian, 1956a). Heukelekian (1956b), showed that for both polyvinyl chloride (PVC) and polyethylene sheets with 3-grades of roughness, the attachment rates and total attached biofilm mass were directly proportional to surface roughness.

## 2.8 CCMB

The CCMB was developed in the early 1990's by John Junius of A-1 Aquaculture, who has successfully patented the design (Junius and Junius, 1996) and has since installed variously sized commercial units in several private and research aquaculture system growing a variety of organisms and even in municipal wastewater treatment systems.

The CCMB was developed after considering the major limitations of all aquaculture nitrification systems (Junius, 1994). However, it is a valuable exercise to trace through the logical development of the CCMB from an in depth literature review.

### 2.8.1 Spouted Bed Evolution

Have to reword a few sentences to make sure I'm not copying directly from Scott et al. (1997).

It has been shown that distinct biomass profiles are present in fluidized beds whereby a steady state exists with thicker biofilm coated particles and thus lower density residing in the upper portion of the bed and lesser coated particles in the lower (Shieh et al., 1981; Eggers and Terlouw, 1979; Nam et al., 2000). Bousfield and Hermanowicz (1984) stated that segregation of this type decreases bioreactor efficiency

since the majority of the biomass is present in the region of lower substrate concentration near the effluent and cannot be fully utilized. They showed that a more homogeneous bed could be maintained with a thinner and more active biofilm throughout, thus increasing substrate utilization up to 30% by employing a mechanism to recycle particles from the top to the bottom of the bed while simultaneously cleaning excess biomass.

Increasing performance by recycling particles within a fluidized bed is not new (Scott et al., 1997). The first paper describing the use of spouted-beds for grain drying appeared almost 50 years ago (Mathur and Gishler, 1955). Since that time, numerous applications for various spouted-bed configurations including granulating, drying, blending, coating, combustion, pyrolysis, gasification, catalytic polymerization (Olazar et al., 1993), crystallization (Palwe et al., 1985), and fixed-film biological processes (Tang and Fan, 1987; Fan et al., 1990; Kennard and Janekeh, 1991; Obradovic et al., 1994) have been described.

The configuration of a traditional cylindrical spouted-bed reactor is very similar to a fluidized bed with two obvious differences (Figure 2.8.1-1). First, flow is applied only to the center of the bed, resulting in a well-defined particle recirculation travelling up through the center and down the sides and utilizing less energy than a fluidized bed (Olazar et al., 1994). Second, the hydrodynamics of spouted-beds impose a limit to the spoutable height, therefore traditional spouted-beds are usually not as tall as fluidized beds (Olazar et al., 1993; Cecen, 1995).

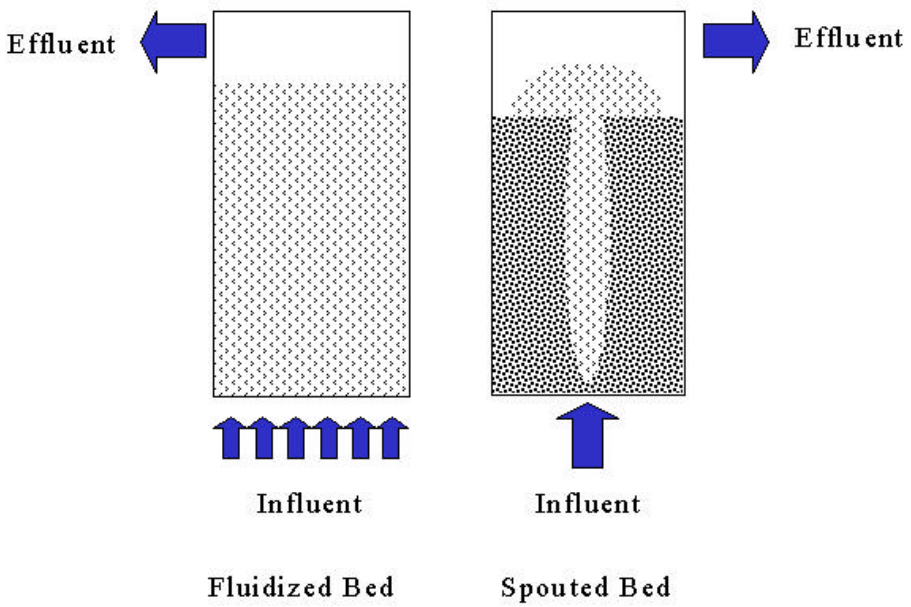


Figure 2.8.1-1. Fluidized bed and spouted bed traditional configurations (after Scott et al., 1997).

The maximum spoutable height limitation was minimized with a central draft-tube (Figure 2.8.1-2) added to the spouted-bed configuration in 1965, and which serves to contain the spout (Grbavcic et. al, 1992; Alappat and Rane, 1995). The draft tube both removed the bed height limitations and improved the particle recycle efficiency up to five times that of a traditional spouted-bed (Grbavcic et al., 1992).

As with aerobic fluidized bed bioreactors, aerobic spouted-bed bioreactors depend upon influent $O_2$ concentrations to meet bacterial respiration requirements (Scott et al., 1997). However, unlike fluidized bed systems significant water flow is recycled along with the cycling media in a spouted-bed (Kim and Littman, 1987; Tang and Fan, 1987). This higher hydraulic retention time coupled with lower flow rates could adversely impact the performance of draft-tube liquid-solid spouted-beds when used as aerobic bioreactors and may explain why spouted-bed aerobic bioreactors were not reported in the literature until the advent of the three-phase (gas-liquid-solid) draft-tube spouted-bed reactor (Scott et al., 1997).

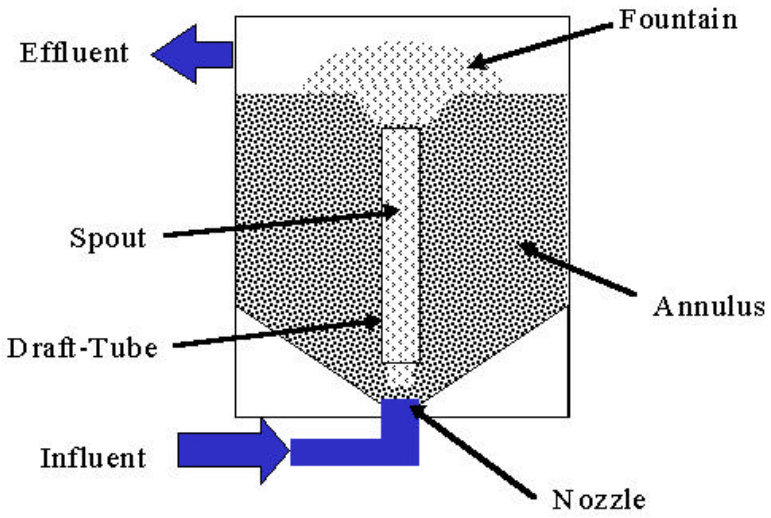


Figure 2.8.1-2. Spouted bed reactor with draft-tube (after Scott et al., 1997).

The three-phase draft-tube spouted-bed reactor configuration known as the jet-spouted bed or jet-loop reactor (Figure 2.8.1-3) is of particular interest. A two-fluid (gas-liquid) nozzle is employed at the bottom of the draft-tube reactor, either with an annular liquid nozzle and inner gas nozzle, or a venturi device (Yagna Prasad and Ramanujam, 1995). Two problems have been described concerning the jet-loop reactor. First, a low residence time of the gaseous phase as injected gasses travel straight up the draft-tube and out. Second, solids can physically block the nozzles. These problems are addressed by reversing flow through the spouted bed reactor (Yagna Prasad and Ramanujam, 1995).

A reversed-flow jet-loop reactor positions the two-fluid nozzle at the top of the reactor (Figure 2.8.1-3). The gas-liquid-solid mixture encounters an impact-plate that forces an abrupt change in direction rather than pluming around the draft-tube exit to form a fountain as in the traditional jet-loop reactor. Furthermore, placement of both the influent and effluent at the top of the reactor eliminate short-circuiting (Padmavathi and Remananda Rao, 1991; Yagna Prasad and Ramanujam, 1995). While the literature

indicates that reversed-flow jet-loop reactors have applications in wastewater treatment, no only gas-liquid mixing and dispersion studies could be located (Scott et al., 1997). One possible explanation could be excessive biofilm detachment, documented to occur when rising air bubbles encounter small diameter particles in a liquid stream and result in gas effervescence (Tanaka et al., 1981; Shieh and Li, 1989). This phenomenon may occur in the annulus of reversed-flow jet-loop reactors (Scott et al., 1997).

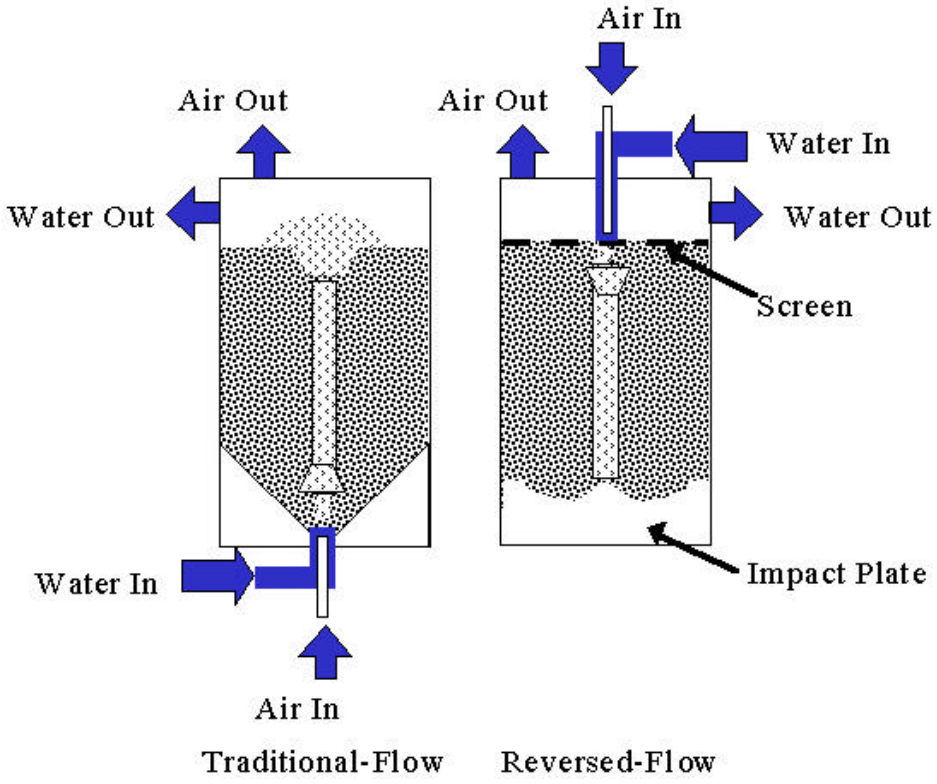


Figure 2.8.1-3. Jet-loop reactor and reversed-flow jet-loop reactor configurations (after Scott et al., 1997).

The Junius and Junius (1996) improvements to the spouted-bed bioreactor keep the better mixing, non-clogging, and no short-circuiting advantages of the reversed-flow jet-loop reactor without the disadvantage of effervescence induced biofilm detachment (Scott et al., 1997). Louisiana based A-1 AQUACULTURE distributes the unit and has referred to this new configuration as the Continuous-Cleaning Multi-Functional

Bioreactor (CCMB). The CCMB is a reversed-flow, concentric-draft, elliptical semi-closed loop, three-phase bioreactor. The patented three-phase interface allows the cycling low-density media to fully ascend above the water-line form biofilm reaeration and fluid $CO_2$ stripping such as in a Biodrum or RBC before descending to the spout and traveling down the draft-tube. The lack of forced air injection minimizes gas effervescence, while the continuously cycling media provide self-aeration.

2.8.2 Solids Removal

The CCMB is termed "multi-functional" as it may be operated either as a primary nitrifying biofilter when the media continuously cycles or as a primary solids capturing device with static media (Lawson et al., 1996). In "static mode" the CCMB may be compared to the expandable granular biofilters (EGBs) sometimes also referred to as "floating bead filters" developed at LSU's Civil Engineering Aquatic Systems Laboratory CEASL and first tested by Wimberly (1990). The notable difference is that the CCMB in "static mode" requires complicated backflushing, retains its full flow rate during use, and is not contained in a pressurized vessel like the "floating bead filter," which slowly clogs during use (Scott et al., 1997). There has been no quantitative research on the static mode of the CCMB. Therefore it is not addressed in this study.

**2.9 Estimating Filter Conversion Rates**

The simplest rate of nitrification that can be calculated for a biofilter is simply the mass conversion rate of nitrogen from one form to another or the gross nitrification rate. The first stage of nitrification or nitritation can be calculated using Equation 2.9-1.

$$C_A = Q_R \left(A_{in} - A_{out}\right)(1440) \qquad (2.9\text{-}1)$$

where

$C_A$ = gross nitritation (TAN to $NO_2^-$-N) rate (mg/day)
$Q_R$ = flow rate through the filter (L/min)
$A_{in}$ = inlet TAN to the filter (mg/L)
$A_{out}$ = outlet TAN from the filter (mg/L)
1440 = conversion factor (min/day)

The second stage of gross nitrification or nitratation ($NO_2^-$-N to $NO_3^-$-N) can be calculated using Equation 2.9-2. The equation is structured such that it captures the mass conversion of $NO_2^-$-N to $NO_3^-$-N attributed both to the observed difference in $NO_2^-$-N across the biofilter and the $NO_2^-$-N generated by $C_A$.

$$C_N = C_A + Q_R\left(N_{in} - N_{out}\right)(1440) \quad (2.9\text{-}2)$$

where

$C_N$ = apparent nitratation ($NO_2^-$-N to $NO_3^-$-N) rate (mg/day)
$N_{in}$ = inlet $NO_2^-$-N to the filter (mg/L)
$N_{out}$ = outlet $NO_2^-$-N from the filter (mg/L)

Although $C_N$ gives a more complete estimate of total nitrification by accounting for the conversion of TAN to $NO_3^-$-N, the first stage (nitritation) is more often reported in the literature alone as it is considered the rate limiting of the two stages (see Section 2.4).

It is often desirable to compare the performance of nitrifying systems not just on their gross nitrification rates, but on their nitrification rates per unit of media volume (volumetric nitrification rate) or per unit of media surface area (areal nitrification rate). This allows systems of varying sizes or capacities to be compared on common terms. Other comparisons could be made such as the nitrification rate per unit of energy consumed, operating cost, or physical footprint.

Volumetric nitrification rates are calculated by dividing gross nitrification rates by the bulk volume of the media. The first stage of volumetric nitrification (nitritation) can be calculated using Equation 2.9-3 (Sastry, 1996; Goltz et al., 1999).

$$C_A^V = \frac{Q_R (A_{in} - A_{out})(1440)}{V_M} \tag{2.9-3}$$

where

$C_A^V$ = volumetric nitritation rate (mg/m$^3$day)
$Q_R$ = flow rate through the filter (L/min)
$A_{in}$ = inlet TAN to the filter (mg/L)
$A_{out}$ = outlet TAN from the filter (mg/L)
$V_M$ = filter bulk media volume (m$^3$)
1440 = conversion factor (min/day)

The second stage of volumetric nitritation or nitratation can be calculated using Equation 2.9-4 (Sastry, 1996).

$$C_N^V = C_A^V + \frac{Q_R (N_{in} - N_{out})(1440)}{V_M} \tag{2.9-4}$$

where

$C_N^V$ = apparent areal nitratation rate (mg/m$^3$day)
$N_{in}$ = inlet $NO_2^-$-N to the filter (mg/L)
$N_{out}$ = outlet $NO_2^-$-N from the filter (mg/L)

Areal nitrification rates are calculated by dividing gross nitrification rates by the surface area of the media. Equations 2.9-3 and 2.9-4 can be used to calculate the areal nitrification rates by multiplying each $V_M$ term by the specific surface area to produce Equations 2.9-5 and 2.9-6.

$$C_A^A = \frac{Q_R (A_{in} - A_{out})(1440)}{V_M S_M} \tag{2.9-5}$$

$$C_N^A = C_A^A + \frac{Q_R\left(N_{in} - N_{out}\right)(1440)}{V_M S_M} \tag{2.9-6}$$

where

$C_A^A$ = apparent areal nitritation rate (mg/m$^2$day)
$C_N^A$ = apparent areal nitratation rate (mg/m$^2$day)
$S_M$ = specific surface area of the filter media (m$^2$/m$^3$)

Because nitrification is a function of N substrate concentration that is generally linear at N substrate concentrations between 0 mg/L and 1 mg/L (Iwai and Kitao, 1994), all of nitrification rates calculated above can be normalized for varying substrate concentrations (< 1 mg N/L) by dividing by the biofilter influent substrate concentrations. Applying this procedure to Equations 2.9-5 and 2.9-6 produces Equations 2.9-7 and 2.9-8.

$$C_A^{A'} = \frac{Q_R\left(A_{in} - A_{out}\right)(1440)}{V_M S_M A_{in}} \tag{2.9-7}$$

$$C_N^{A'} = C_A^{A'} + \frac{Q_R\left(N_{in} - N_{out}\right)(1440)}{V_M S_M N_{in}} \tag{2.9-8}$$

Alternately, Sastry (1996) presented a means of normalizing the nitrification rates by dividing by the average between the filter influent and effluent concentrations.

# CHAPTER 3

# METHODS AND MATERIALS

Three identical recirculating aquaculture systems (RAS) were constructed at the Louisiana State University Agricultural (Ag) Center Ben Hur Aquacultural Research Facility within an indoor laboratory. To meet the objectives of this study each system utilized a different plastic filter media and the solids removal scheme was altered over 3 steady-state trials to achieve increasing organic loads against which to evaluate the nitrification rates of each system. A history of experiments conducted is provided in Table 3-1.

Table 3-1. Experimental trial definitions.

| Trial | Solids-Removal Device | Fish Feed Rate g/day (lbs/day) | Flow Rate lpm (gpm) | System Volume L (gal) | System Daily Exchange Rate | N |
|---|---|---|---|---|---|---|
| 1 | Settling basin with tube-settler media | 400 (0.88) | 56.14 (14.83) | 4,840 (1,279) | 6.07% | 5 (over 5 days) |
| 2 | Settling basin with tube-settler media | 1,000 (2.2) | 56.14 (14.83) | 4,840 (1,279) | 5.62% | 5 (over 5 days) |
| 3 | Settling Basin alone | 1,000 (2.2) | 56.14 (14.83) | 4,840 (1,279) | 5.62% | 5 (over 5 days) |
| 4 | None | 1,000 (2.2) | 56.14 (14.83) | 3,874 (1,023) | 7.02% | 6 (over 24 hrs) |
| 5 | None | 1,000 (2.2) | 80.7 (21.32) | 3,874 (1,023) | 7.02% | 6 (over 24 hrs) |

## 3.1 Recirculating Systems

Pilot-scale, 4,840 L (1,280 gal) water volume, RAS with CCMB media bulk volumes of ~0.12 $m^3$ (4.27 $ft^3$) were chosen for a combination of real-world scalability, laboratory space, and component availability. To ensure that the pilot-scale RAS were capable of sustaining the fish stock through the duration of the study and that the results

of this research could be applied to commercial RAS, the experimental systems were assembled with all the components of commercial RAS. A schematic diagram representing the three pilot-scale systems is shown in Figure 3.1-1.

3.1.1 CCMB

Three CCMB units were designed and fabricated specifically for this research from proprietary translucent fiberglass sheeting provided by A-1 Aquaculture and standard aluminum stock using larger units commercially offered by A-1 Aquaculture as a guide. Each CCMB contained 0.121 $m^3$ (4.27 $ft^3$) of granular/pellet plastic media with the characteristics shown in Table 3.1.1-1. Scanned images of each media type are shown in Appendix F. The units held a gross reactor volume of 0.200$m^3$ (7.07 $ft^3$) or 200 L (52.9 gal), however the actual liquid volume of each reactor varied slightly with the porosity of each media (see Table 3.1.1-2). An additional liquid volume of 55.6 L (14.7 gal) was contained in each CCMB effluent manifold for a total gross liquid volume of 256 L (67.6 gal) or total actual liquid volume (average between the 3 systems) of 213 L (56.3 gal).

Table 3.1.1-1. CCMB media characteristics.

| Media | Color | Material | Porosity % | Specific Gravity | Specific Surface Area[1] $m^2/m^3$ ($ft^2/ft^3$) | Sphericity % | Volume $cm^3$ ($x10^{-3}$ $ft^3$) |
|---|---|---|---|---|---|---|---|
| 1 | Grey | Recycled Polypropylene | 34.9 | 0.841 | 1,094 (333) | 83.7% | 82.2 (2.90) |
| 2 | White | Polyethylene | 33.2 | 0.874 | 1,155 (352) | 86.7% | 66.8 (2.36) |
| 3 | Black | Post-Consumer Recycled Polypropylene | 38.2 | 0.819 | 1,219 (372) | 82.3% | 72.7 (2.57) |

[1]Apparent Specific Surface Area does not account for random holes (i.e., divots, channels, indentations) in the media that increase the actual surface area but may be unavailable to nitrifiers as they clog or biofoul.

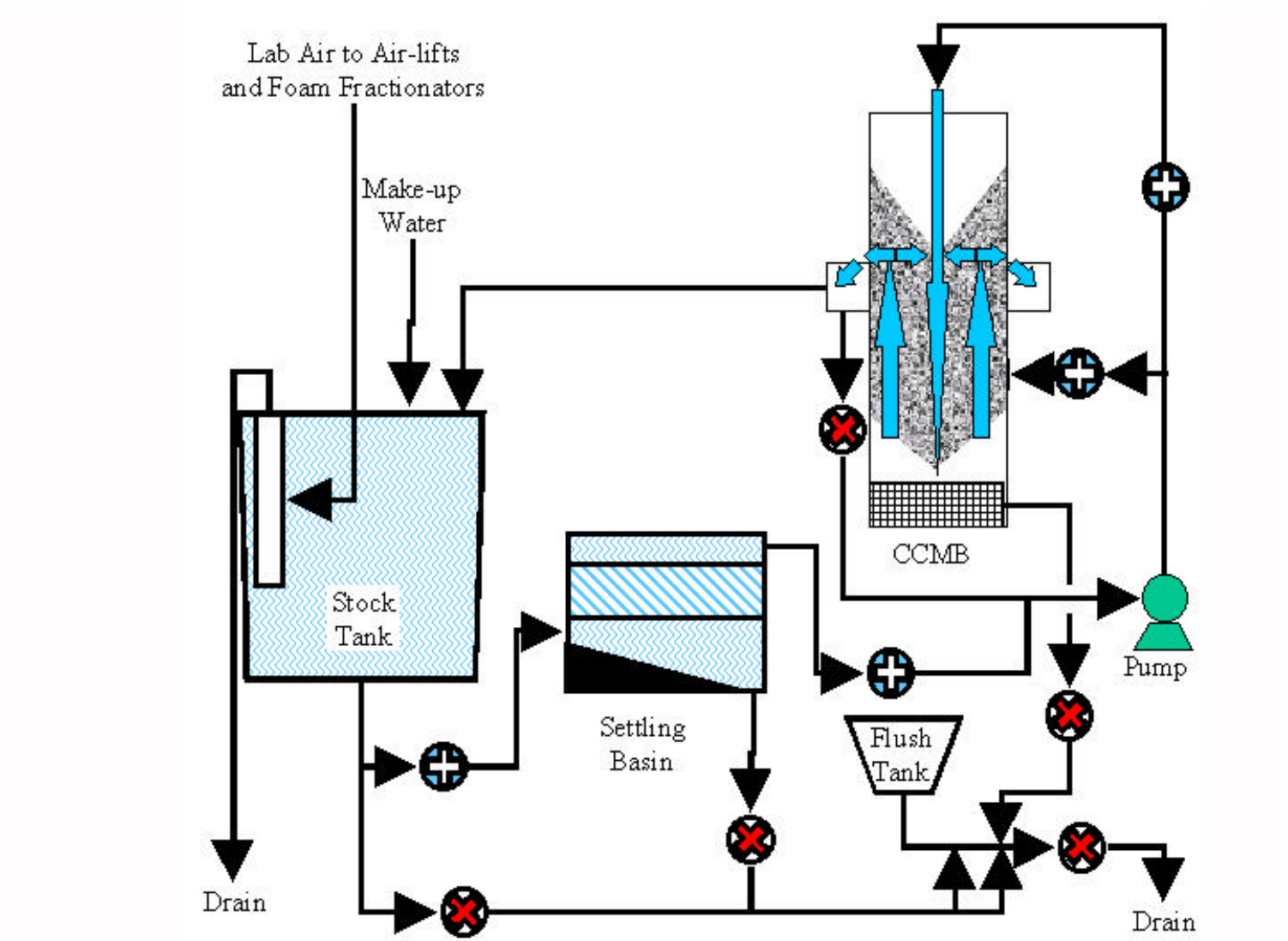


Figure 3.1-1. Experimental systems schematic.

Table 3.1.1-2. CCMB media related system reactor characteristics.

| System | Media | Media Surface Area $m^2(ft^2)$ | Reactor Volume[2] L (gal) |
|---|---|---|---|
| 1 | 1 | 132(1,424) | 154 (40.7) |
| 2 | 2 | 140(1,504) | 160 (42.3) |
| 3 | 3 | 147(1,587) | 158 (41.7) |

[2]Actual liquid volume of the reactor portion of the CCMB units containing media of the porosities shown in Table 3.1.1-1.

The major components of the CCMB units included (Figure 3.1.1-1) the primary influent nozzle, secondary influent line, low-density granular plastic media, media screen, reactor tank, effluent manifold, draft-tube, and drain port.

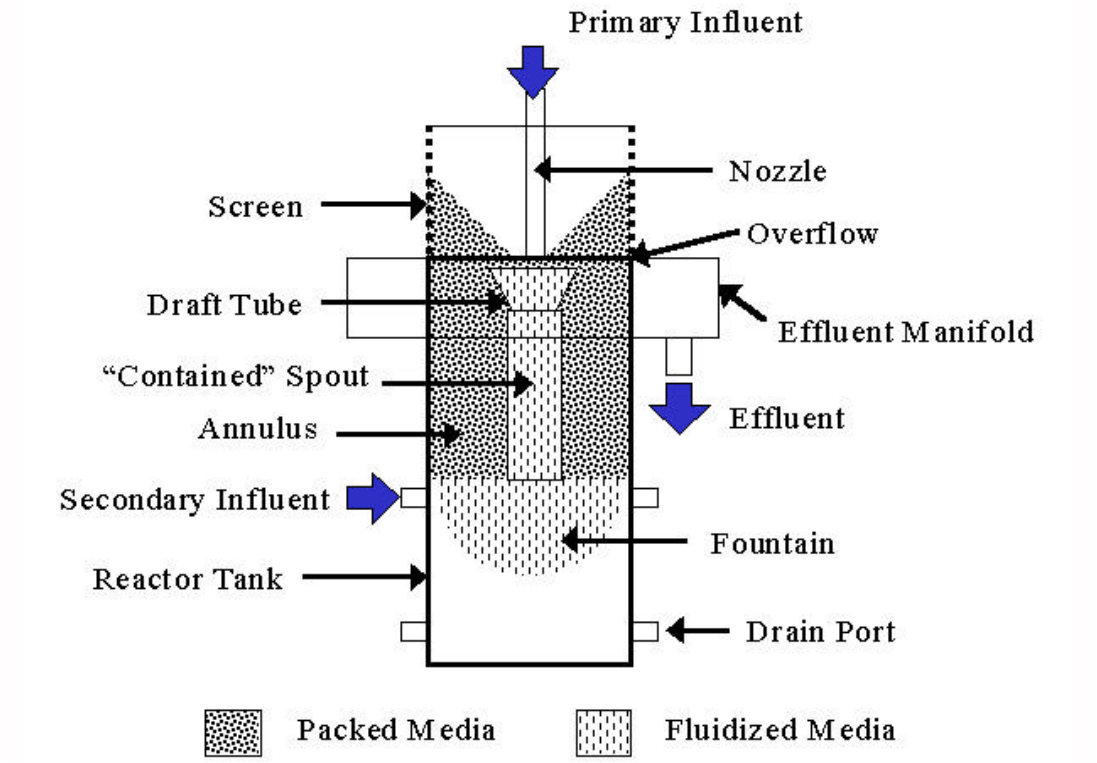


Figure 3.1.1-1. CCMB components (after Scott et al., 1997).

In each system, water flowed to the CCMB from the discharge of the recirculating pump. The majority of the flow entered the CCMB through the primary influent nozzle located at the top of the unit and a lesser amount through the secondary influent line located midway down the unit on its side (Figure 3.1.1-1) to slow the internal media

rotation cycle. After cycling the media, water exited the CCMB screen to the effluent manifold from which it flowed by gravity to the culture tank.

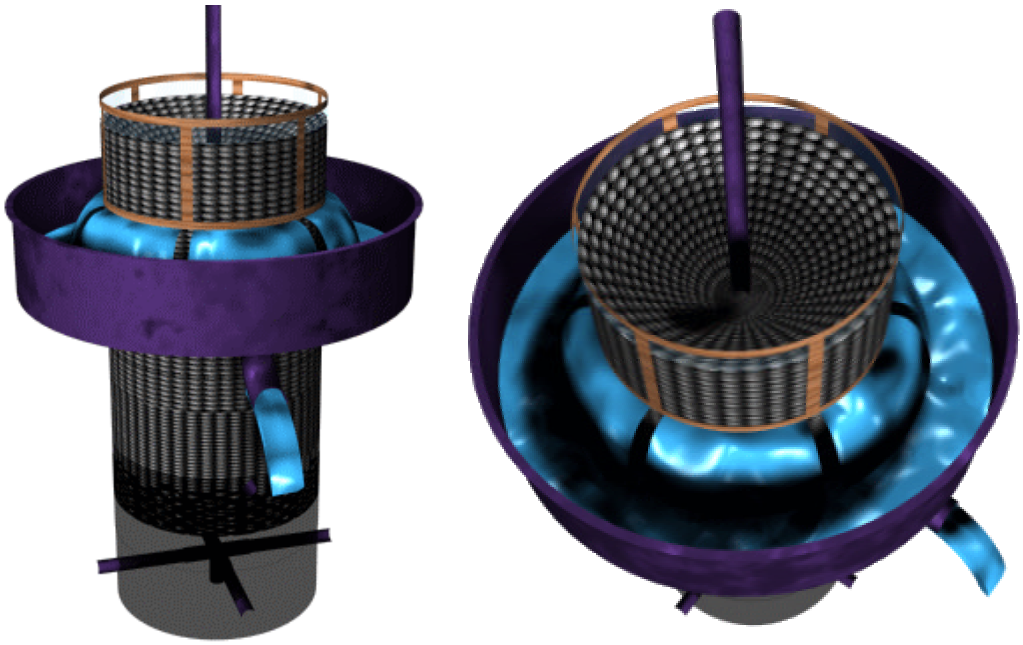

Figure 3.1.1-2. CCMB three-dimensional views (courtesy A-1 Aquaculture).

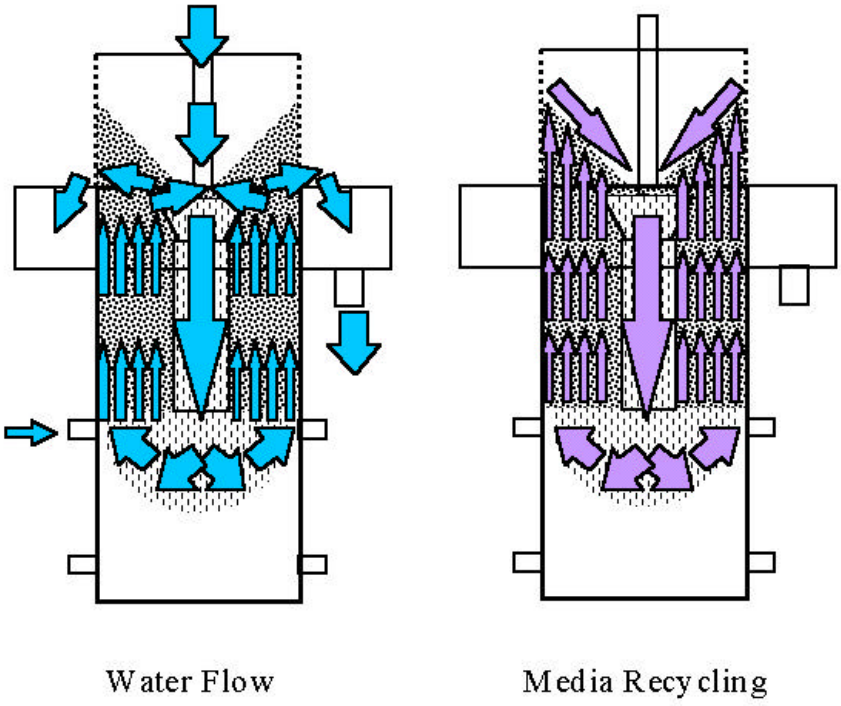


Figure 3.1.1-3. Water flow and media recycling patterns (after Scott et al., 1997).

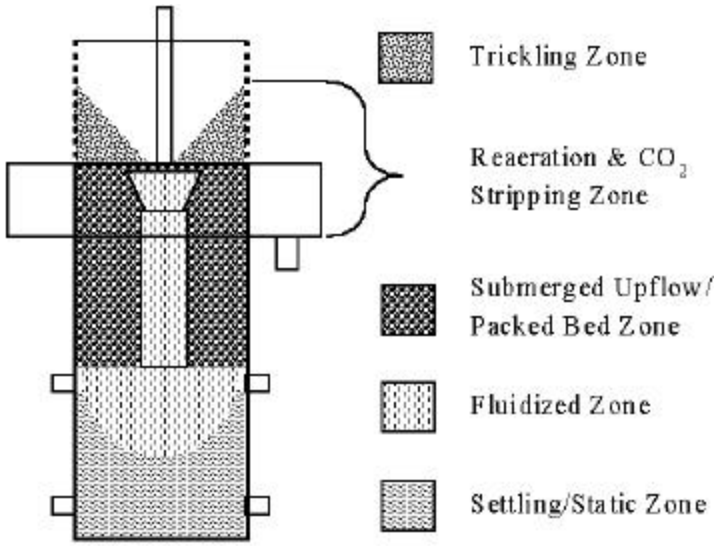


Figure 3.1.1-4. CCMB zones analogous to traditional water treatment processes (after Scott et al., 1997).

Constant media and water recycling (Figure 3.1.1-3) within the CCMB produce the mixing, aeration, and shear required for efficient nitrification without biofouling. Although the water flow and media are commingled during cycling, the CCMB may be broken down into zones (Figure 3.1.1-4) analogous to traditional water treatment processes. These include the fluidized zone, settling/static zone, submerged-upflow/packed-bed zone, trickling zone, and reaeration and $CO_2$ stripping zone (Scott et al., 1997).

3.1.1.1 Fluidized Zone

Under normal operating conditions, nearly all water to be treated enters the CCMB through the primary influent nozzle. As this water encounters the bed of low-density floating media, it produces a low-pressure fluidized spout that transports media down the draft-tube. After the spout clears the lower edge of the draft-tube, the water flow travels back up the annulus of the unit as the media momentarily hovers in a pattern

similar to an inverted fountain before floating upwards and compressing into a packed bed annulus (Scott et al., 1997).

The CCMB has many of the advantages of a fluidized bed biofilter without the disadvantages. Due to the media screen, there is no loss of media as in fluidized sand filters. Also, pumping costs could be less than for fluidized sand filters because (Scott et al., 1997):

1. the difference in density between the media and water is less than that between sand and water, therefore less energy would be required for fluidization;
2. only the center of the bed requires fluidization versus the entire cross-sectional area, and;
3. the CCMB is not pressurized, therefore headlosses are predominantly from plumbing to the unit.

3.1.1.2 Settling / Static Zone

Water exiting the draft-tube, flows partially through the fountain region before all returning through the annulus. This creates "dead" zone in which heavy particulates may settle to the bottom of the reactor tank. Draining these solids daily versus weekly showed no discernable impact on nitrification (Scott, et al., 1997).

The static zone becomes anaerobic with time. Findings at the University of Arizona show a slight reduction in $NO_3^-$-N across the CCMB, possibly indicating that some degree of anaerobic denitrification occurs in the static zone (Fitzsimmons, 1997).

3.1.1.3 Submerged-Upflow/Packed-Bed Zone

As the media particles ascend from the fountain, they impact a bed of packed media within the annulus. Four primary forces promote the formation of this packed bed.

1. The downward force of media already risen above the overflow water-line (trickling zone) in the screen.
2. The downward force exerted by the flange at the top of the draft-tube.
3. The buoyant force of rising media from the fountain.
4. The upward force exerted by water moving, up and through, the packed bed.

These combined forces, cause the entire packed bed to proceed upwards as a single unit at a rate of about one foot per minute. Permitting a fraction of the total influent flow to enter the unit via the secondary inlet; increases the force of water pushing through the annulus, further compresses the packed bed, and retards the media and water cycling speeds (Scott, et al., 1997). Compacting the bed allows the media to rise further out of the water at the top of the cycle. The effect of media cycle speed on filter performance is unknown.

3.1.1.4 Trickling Zone

As the packed bed continuously emerges above the screen overflow water-line, it shears off along approximately a 45 degree angle of repose as the media particles descend towards the center and into the spout. Some unknown fraction of the water flowing up through the annulus flows with the media into the spout, while most flows out of the reactor tank via the screen (Scott, et al., 1997).

Media above the overflow water-line forms a trickling filter. The trickling action is theoretically very similar that encountered in a RBC or Biodrum, where clinging water trickles from media rising from submersion (Scott, et al., 1997).

3.1.1.5 Reaeration & CO2 Stripping Zone

The upper portion of the CCMB provides the air-water contact required for reaeration and $CO_2$ stripping. These actions occur in the trickling zone, as the effluent flows through the media screen to the effluent manifold via sheet-flow, and where the effluent gravity feeds from the effluent manifold back to the system (Scott, et al., 1997).

Sheet-flow is an efficient mechanism for providing air/water gas exchange. All water exiting the CCMB undergoes sheet-flow across the 30 cm (1 ft) drop from the screen to the floor of the effluent manifold. In addition, depending upon the path by which water exits the effluent manifold, sheet flow can be designed to occur between the effluent manifold and the receiving tank (Scott, et al., 1997).

3.1.2 Rearing Tank

Circular gel-coated fiberglass tanks each having a diameter of 2.3 m (7.54 ft) and height of 0.914 m (3 ft) for a total volume of 3,790 L (1,000 gal) were utilized as the rearing tanks. The tank walls were slightly tapered and the floor was slightly sloped towards a center 7.62 cm (3 in) bottom drain. Each rearing tank was filled to a depth of 0.878 m (2.88 ft) for a working volume of 3,637 L (961 gal). As this level was very near the top of the tanks, a 0.914 m (3 ft) tall plastic-coated wire mesh vertical fence was erected around the perimeter of each tank.

In each system, effluent from the CCMB flowed by gravity from the CCMB effluent manifold to the rearing tank via a 5.08 cm (2 in) diameter horizontal effluent

spray bar extending through the side of the CCMB effluent manifold (slightly overlapping the edge of the culture tank) and extending to the center of the rearing tank. The desired flow distribution was achieved by careful sizing, spacing, and direction of the flow-holes drilled into the spray bar. Water exited the rearing tank through a 7.62 cm (3 in) vertical center stand-pipe extending from the bottom of the tank to the water surface and drilled with variably-spaced and sized holes to promote the desired full-tank flow pattern. The stand-pipe was wrapped with vinyl-coated mesh spaced 15.2 cm (6 in) away from the pipe in order to prevent dead or dying fish from plugging the stand-pipe. Water flowed from the stand-pipe through the center drain, which was plumbed to a drain-pipe that ran underneath the tank to an outer skirting that supported the tank above the floor.

3.1.3 Settling Basin / Tube Settlers

Three settling basins were constructed of marine-grade plywood, supported by pine frames, and lined with fiberglass sheeting. Each 124 cm (49 in) square by 76.2 cm (30 in) deep basin was fitted with a 124 cm (49 in) by 110.5 cm (43.5 in) sealed false-floor sloped 16.3 % towards a submerged manifold drained via one 5.08 cm (2 in) pipe installed through the bottom of the basin and designed to remove solids evenly from the floor of the basin during flushes. Each basin was operated with a water level of 70.2 cm (27.6 in) for a working water volume of 966 L (255 gal).

Tube settler trials utilized four rectangular blocks of 30.5 cm (12 in) square by 122 cm ( 48 in) Bio Strata media (Part Number LS42A, 138 $m^2/m^3$ (42 $ft^2/ft^3$), Aquatic Eco-Systems, Inc., Apopka, FL), in each settling basin. Bio Strata media consists of ribbed corrugated black PVC glued into a block to form tubes crisscrossing at 60 degrees to the

horizontal. The blocks were suspended horizontally at 9.14 cm (3.6 in) below the settling basin water surface.

For trials utilizing either settling basins or tube settlers, water flowed by gravity from the 7.62 cm (3 in) rearing tank drain-pipe to the settling basin where two 5.08 cm (2 in) pipes split the flow and carried it through the side of the settling basin and into a horizontal distribution manifold located just above the top of the sloped false-bottom (the shallow end) and constructed by drilling variable sized and spaced holes in a 5.08 cm (2 in) diameter 122 cm (48 in) long PVC pipe. The flow then traveled up and across the settling basin to a similarly constructed horizontal effluent manifold located at the settler wall and between the top of the tube settlers and the water surface (the deep end). From this manifold, water exited through the side (opposite the influent manifold) of the settling basin via two 5.08 cm (2 in) pipes, which converged before entering the recirculating pump intake.

For trials with no solids removal device, the valve and piping configuration allowed the settling basins to be completely bypassed.

### 3.1.4 Water Recirculation

Water recirculation in each system was performed by a 0.373 kW (0.5 hp) driven bronze centrifugal pump (Model 1P996) with an influent strainer basket (Model 1P999), both manufactured by TEEL Water Systems, Chicago, IL.

During Trials 1 through 4, flow was maintained at 56.14 lpm (14.83 gpm) and increased for trial 5 to 80.7 lpm (21.32 gpm). Flow adjustments were made by adjusting a 5.08 cm (2 in) ball-valve between the recirculating pump discharge and the CCMB. Once the spray bar was reinstalled the top of the water level miniscus in the clear-fiberglass CCMB effluent manifold was marked. Subsequent flow adjustments

were made as needed and always before analytical samples were taken to return the miniscus to this mark. Confirmatory flow measurements were initially conducted daily and later weekly to show the reliability of the miniscus in predicting flow from the gravity-drained CCMB effluent manifold.

### 3.1.5 Aeration and Dissolved Solids Removal

Laboratory air was supplied by a single, remotely located, continuously operating, 1.12 kW (1.5 hp), regenerative blower (Model DR454R72), manufactured by ROTRON, Inc., Woodstock, NY. Each rearing tank contained two airlifts constructed of 7.62 cm (3 in) PVC pipe and fittings with a single 3.81 cm (1.5 in) square by 7.62 cm (3 in) long glass bonded silica diffuser (airstone) distributed by Aquatic Eco-Systems, Apopka, FL.

For dissolved solids removal, a single countercurrent foam fractionator, described by Lawson (1995) was constructed of 15.2 cm (6 in) and 7.62 cm (3 in) diameter PVC pipe and fittings utilizing two of the above mentioned airstones and was placed in each rearing tank.

The two airlifts and single foam fractionator were attached to the outer wall of each tank at equal distances. The flow from each was pointed in the same direction but aimed so as not to dramatically increase the rotation of the culture water.

### 3.1.6 Water Exchange and Solids Removal

All water-containing components in each system were connected to that system's single main drain line. Each component drain-line was individually valved-off from the system main drain line that was in turn also valved-off from the under-floor laboratory drain. Between the component valves and the system main drain valve, the line was hydraulically connected to a 303 L (80 gal) fiberglass waste tank.

The waste tank served three purposes. The primary purpose of the waste tank was to quantify the volume of water removed from the system during each water exchange / solids removal event. Secondly, the waste tank provided a convenient mechanism to sterilize the waste-water via addition of and thorough mixing with hypochlorite ($ClO^-$) to reduce the likelihood that Tilapia eggs or fry would escape into the environment. Finally, by keeping all component valves and the system main drain valve in the closed position while not in use, the system water level could drop no further than the level of the top of the waste tank should any component valve leak or be left open. This measure was needed since the rearing tank stand pipe had been perforated for flow distribution and could not provide the safety of a traditional overflow drain.

During Trials 1, 2, and 3, system water exchanges were performed on each system once per day by hydraulically bypassing the settling basin; shaking the tube settler media (Trials 1 and 2 only); and opening the settling basin component drain valve until clear water flowed into the waste tank. The drain ports on each CCMB were also opened briefly and on an intermittent basis During Trials 1, 2, and 3 to allow solids accumulated at the bottoms of the CCMB units to exit. These drains were utilized as the sole flush point for Trials 4 and 5 since the settling basins had been completely bypassed. During Trial 1 the flush volume, which had been set at 294 L (77.75 gal), was observed to be more than the minimum amount required to adequately flush accumulated solids from the bottom of the settling basins, therefore the flush volumes for Trials 2 through 5 were set at 272 L (71.75 gal). This amount equals 5.62 % of the total system water volume or 7.48 % of the rearing tank water volume exchanged daily during Trials 2 through 5. Because the settling basins were removed for Trials 4 and 5

and the flush volumes remained the same, the exchange rate of the total system water volume increased to 7.02 % per day. Generally, systems with daily water exchange rates of up to 10% of the total system water volume are considered recirculating systems (Huguenin and Colt, 1992; Lawson, 1995) and can be called "closed systems" (Losordo et al., 1992). Advances in $NO_3^-$ and phosphorus (P) removal with anoxic bioreactors has only recently demonstrated the technical feasibility for the long-term operation of zero-discharge RAS (Shnel et al., 2002).

Table 3.1.6-1. System water volumes and exchange rates by trial.

| Trial | Volume | | Exchange Rate | |
|---|---|---|---|---|
| | Total System L (gal) | Flush Water L (gal) | Total System | Culture Tank |
| 1 | 4,840 (1,279) | 294 (77.75) | 6.07% | 8.08% |
| 2 | 4,840 (1,279) | 272 (71.17) | 5.62% | 7.48% |
| 3 | 4,840 (1,279) | 272 (71.17) | 5.62% | 7.48% |
| 4 | 3,874 (1,023) | 272 (71.17) | 7.02% | 7.48% |
| 5 | 3,874 (1,023) | 272 (71.17) | 7.02% | 7.48% |
| Average | 4,453 (1,176) | 276 (72.91) | 6.27% | 7.60% |

3.1.7 Environmental Control

Temperature was maintained near the 25 °C (77 °F) recommended in Section 2.5.2 by utilizing the laboratory air conditioning, venting, and heating systems. Periodic additions of sodium bicarbonate ($NaHCO_3$) were used to control pH and Alkalinity to the ranges specified in Section 2.5.3 and Section 2.5.4, respectively.

**3.2 Systems Startup**

Following a successful startup and shakedown of the mechanical systems each CCMB with its recirculation pump was hydraulically isolated from the rest of each RAS and artificially acclimated similar to the methods described by Manthe and Malone (1987) and DeLosReyes (1995). The purpose of the isolation was to ensure that the

CCMB units were acclimated and not every surface area in each RAS, namely the tube-settler media. On January 5th, 1998, each system was spiked with ammonium chloride ($NH_4Cl$) and sodium nitrite ($NaNO_2$) to yield approximately 12.9 mg/L TAN and 5.15 mg/L $NO_2^-$-N. This was followed by the addition to each system of approximately 5.5 grams of dried bacteria and 0.5 grams of concentrated live bacteria from a specialized experimental A-1 Aquaculture CCMB used to produce concentrated nitrifying bacteria. No water exchanges were performed during the entire acclimation period.

**3.3 Stocking and Feeding**

On February 5, 1998 (day 32), 611 juvenile, hormone treated, sex-reversed, and electrophoretically certified tilapia (*Oreochromis niloticus*) weighing an average of 208 g (0.459 lb) each were transported from the Til-Tech Aqua Farm in Robert, LA to the LSU Ag Center Ben Hur Aquaculture Research Facility south of Baton Rouge, LA along the route authorized by an Inland Fisheries Division of the Louisiana Department of Wildlife and Fisheries (LDWF) approval letter dated February 4, 1998. Upon arriving at the LSU Ag Center facility (LDWF Tilapia Culture Permit Number TL-02R-93), the tilapia were weighed and sorted to provide an even weight distribution between the three systems (see Table 3.3-1).

Table 3.3-1. Initial and tilapia stocking densities.

| System | Total | | Average | Stocking Density | |
|---|---|---|---|---|---|
| | Number of Fish | Weight kg (lb) | Weight kg/fish (lb/fish) | Total System kg/L (lb/gal) | Culture Tank kg/L (lb/gal) |
| 1 | 200 | 42.35 (93.37) | 0.212 (0.467) | 0.00875 (0.0730) | 0.0116 (0.0972) |
| 2 | 206 | 42.45 (93.59) | 0.206 (0.454) | 0.00877 (0.0732) | 0.0117 (0.0974) |
| 3 | 205 | 42.40 (93.48) | 0.207 (0.456) | 0.00876 (0.0731) | 0.0117 (0.0973) |
| Total | 611 | 127.2 (280.4) | 0.208 (0.459) | 0.00876 (0.0731) | 0.0117 (0.0973) |

Nitrogen input into the system consisted solely of fish feed. The fish were fed a 35% protein diet of Clover brand floating catfish fingerling pellets (35% Fingerling

Feed) manufactured by SF Services, Inc. Feed Division, North Little Rock, AR. The feed was delivered to the culture tanks at fixed intervals throughout the day with calibrated vibratory feeders (Model AF7) controlled by a 12 V digital timer (Model SF41 - now discontinued) both manufactured by Sweeney Enterprises of Boerne, TX (http://www.sweeneyfeeders.com). The feeding schedule for Trial 1 consisted of four feedings of 100 g (0.220 lb) delivered at 0600, 1000, 1400, and 1800 hours for a total of 400 g (0.882 lb) to each culture tank. Trials 2 through 5 were provided 10 feedings of 100 g (0.220 lb) delivered at 1.5 hour intervals between 0600and 1930 hours for a total of 1,000 g (2.20 lb) to each culture tank.

**3.4 Methods of Analysis**

3.4.1 Water Quality

Water quality sampling and analyses were conducted daily for Trials 1, 2, and 3 and every 4 hours over a 24-hour period for Trials 4 and 5. Temperature, dissolved oxygen (DO), and pH were measured in-situ; TAN, $NO_2^-$-N, $NO_3^-$-N, and alkalinity were measured immediately after collection; and additional samples were preserved in 500 ml Nalgene sample bottles for total suspended solids (TSS) and 10 ml capped vials for COD, both of which were conducted after the conclusion of the trials. CCMB influent sampling and analyses were conducted at the effluent of the settling basin (Trials 1 through 3) or the culture tank (Trials 4 and 5). CCMB effluent sampling and analyses were conducted in the CCMB effluent manifold at the horizontal effluent spray bar bulkhead connector.

3.4.1.1 Dissolved Oxygen

In-situ DO analyses were conducted in the CCMB influent, CCMB effluent, and culture tank (same as CCMB influent for Trials 4 and 5) for all trials and in the CCMB

draft tube and annulus for Trials 2 through 5. DO analyses were performed using a Mackereth cell-type, membrane-covered, polarographic-sensor, probe and meter (OxyGuard Handy Model Mk I - now discontinued) manufactured by OxyGuard International A/S of Birkerød, Denmark (http://www.oxyguard.dk) and distributed by Point Four Systems Inc. of Richmond, British Columbia (http://www.pointfour.com). The measurement of DO using this type of probe is described in Standard Method 4500-O G (APHA, 1998). The unit was calibrated before each use and operated according to the manufacturer's instructions.

3.4.1.2 pH

In-situ temperature and pH analyses were conducted in the CCMB effluent for all trials using a digital pH/temperature/mV/ORP probe and meter (Digi-Sense® Model 5938-10) distributed by the Cole-Parmer Instrument Co. of Vernon Hills, Illinois (http://www.coleparmer.com). The measurement of pH using this type of probe is described in Standard Method 4500-$H^+$ B (APHA, 1998). The pH meter was calibrated before each use and operated according to the manufacturer's instructions.

3.4.1.3 Total Ammonia Nitrogen

TAN analyses were conducted immediately after sample collection from the CCMB influent and effluent using a gas-sensing $NH_3$ ion selective electrode (Catalog No. 13-620-504) manufactured by Fisher Scientific International Inc. of Pittsburgh, PA (http://www.fisherscientific.com). The measurement of TAN using this type of probe is described in Standard Method 4500-$NH_3$ F (APHA, 1998). The unit was calibrated before each trial and operated according to the manufacturer's instructions.

3.4.1.4 Nitrite, Nitrate, and Alkalinity

CCMB influent and effluent samples for $NO_2^-$-N and $NO_3^-$-N, and CCMB effluent samples for alkalinity were analyzed immediately after collection using a portable water quality laboratory (Model DREL/2000 with DR 2000 direct reading portable spectrophotometer) manufactured by the HACH Company of Loveland, Colorado (http://www.hach.com) and kept on-site. All samples for $NO_2^-$-N were analyzed in triplicate using the DR 2000 and HACH Method 8507 (HACH Company, 1997), which is USEPA approved for reporting wastewater compliance monitoring (Federal Register, 1979) and is a colorimetric method similar to Standard Method 4500-$NO_2^-$ B (APHA, 1998). Similarly, all samples for $NO_3^-$-N were also analyzed in triplicate using the DR 2000 and HACH Method 8039 (HACH Company, 1997), which is a cadmium reduction colorimetric method following the same principals as Standard Method 4500-$NO_3^-$ E (APHA, 1998). The analysis for total alkalinity was performed once using the DREL/2000 Digital Titrator and HACH Method 8203 (HACH Company, 1997), which is a sulfuric acid ($H_2SO_4$), phenolphthalein, bromcresol green method following the principals of Standard Method 2320 B (APHA, 1998). The operation, maintenance, and calibrations of the portable laboratory were performed in accordance with the manufacturer's instructions.

3.4.1.5 Chemical Oxygen Demand

Samples collected for COD analysis from the CCMB influent and effluent were preserved at the Department of Biological and Agricultural Engineering (BAE) Animal Waste Laboratory on the LSU Baton Rouge campus until the end of the trials. Analyses were performed using the HACH DR 2000 spectrophotometer, HACH COD Digestion

Reactor with custom digital temperature control, and HACH Method 8000, which is USEPA approved for reporting wastewater compliance monitoring (Federal Register, 1980) and is a colorimetric dichromate reactor digestion method based on a simplification of Standard Method 5220 D (APHA, 1998).

3.4.1.6 Total Suspended Solids

After the conclusion of the experimental trials, TSS analyses were conducted on refrigerated CCMB influent and effluent samples stored at the LSU BAE Department Animal Waste Laboratory using Standard Method 2540 D (APHA, 1998).

3.4.2 Flow

Flow rates from the recirculating pump to the CCMB were calculated by removing the spray bar from the CCMB effluent manifold and replacing it with a solid pipe discharging to a calibrated container and measuring the container fill-time with a stop watch.

3.4.3 Media Dimensions

Upon close inspection, the average pellet of each media type most closely resembled the geometric shape of a cylinder. The average surface area per media pellet was determined by measuring two diameters and a height for 100 pellets using a dial caliper (see Appendix D) and calculating the average surface area of equal size cylinders. The specific surface area (area per unit volume) was determined by counting the number of pellets contained in 500 mL ($cm^3$) and applying the average surface area to that number. Because each media type was initially somewhat hydrophobic, the porosity and specific gravity for each was measured by packing the pellets into a calibrated WHEATON Biochemical Oxygen Demand (BOD) Bottle and then

measuring the liquid volume required to completely fill and the change in mass after filling the remainder of the bottle with pure methanol ($CH_3OH$). This procedure was repeated 3 times for each media.

### 3.5 Experimental Setup

The objectives of this study were met by evaluating the steady-state CCMB performance for three media types simultaneously over successive trials with varying organic loading rates. This was achieved by modifying the experimental RAS configurations to have different solids-removal mechanisms for Trials 2, 3, and 4. Trial 2 utilized a settling basin with tube-settler media; in Trial 3 the settling basin was used without the tube-settler media; and in Trial 4 the settling basin was completely bypassed leaving the experimental systems without a traditional solids removal device, although some solids settling within the bottom of the CCMB was observed and is discussed later.

Two additional trials were conducted outside the original scope of this project. The first out-of-scope trial (Trial 1) was performed at a lower feed rate, the first of three feed rates designed to achieve the objective of three organic loading levels. However, problems with solids removal beginning with Trial 2 (the second planned feed rate) forced a shift in strategy from three feed rates to three solids removal schemes. The second out-of-scope trial (Trial 5) was performed at a second (higher) flow rate as a potential guide to future optimization trials. Table 3-1 illustrates the conditions and components defining each trial.

# CHAPTER 4

# RESULTS AND DISCUSSION

## 4.1 Trial Execution

### 4.1.1 Steady-State Determinations

The point at which steady-state conditions were achieved during each trial was determined by evaluating CCMB influent TAN and $NO_2^-$-N concentrations. When TAN and $NO_2^-$-N concentration levels appeared to have leveled off or were cycling between levels already observed during the current trial, the system was declared to be at steady-state. The system parameters of temperature, DO, pH, and alkalinity were also considered during steady-state determinations as changes in these parameters could be used to explain changes in TAN and $NO_2^-$-N that would otherwise have made the steady-state determinations more difficult. Graphs illustrating TAN, $NO_2^-$-N, temperature, DO over the course of each steady-state determination are included in Appendix A. $NO_3^-$-N influent values measured during each steady-state run are also shown in each graph. The slight increasing trend in $NO_3^-$-N over time was not a factor in determining steady-state as $NO_3^-$-N was expected to accumulate in the systems over time (Lawson, 1995). Alkalinity and pH values were held fairly steady during each trial and were not graphed for purposes of clarity. The increased variability observed during Trial 2 was attributed to additional bacterial and fish adjustments from the 2.5 times increase in the feed rate. All data are given in Appendix E.

### 4.1.2 Trial Length and Paired Data Evaluations

Comparison of means and paired T-tests were performed for each CCMB paired influent and effluent data set (TAN, $NO_2^-$-N, $NO_3^-$-N, COD, DO, and TN). Table 4.1.2-1 shows the mean difference between influent and effluent data for all comparisons of

means significant above a 95% level of error ($p<0.05$). Complete statistical results are given in Appendix B.

Table 4.1.2-1. Significant comparison of means (influent - effluent in mg/L).

| Media | Trial | TAN | $NO_2^-$-N | $NO_3^-$-N | COD | DO | TN |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 0.1946 | | | | -0.436 | |
| | 2 | 0.2206 | | | 4.8 | 0.208 | |
| | 3 | 0.3194 | 0.0214 | | | 0.556 | |
| | 4 | 0.2008 | 0.033 | | -5.167 | 0.382 | |
| | 5 | 0.1642 | 0.0245 | | | | |
| | 2+3 | 0.2700 | 0.0435 | | | 0.382 | |
| 2 | 1 | 0.1626 | | | | -0.304 | |
| | 2 | 0.1790 | | | | | |
| | 3 | 0.2108 | | | | 0.438 | |
| | 4 | 0.1670 | | | | 0.465 | |
| | 5 | 0.1480 | | | | | |
| | 2+3 | 0.1949 | | | | 0.269 | |
| 3 | 1 | 0.1972 | | | | -0.174 | |
| | 2 | 0.1654 | | | | | |
| | 3 | 0.2512 | 0.0998 | | | 0.642 | |
| | 4 | 0.1450 | 0.0468 | | 8.1667 | 0.253 | |
| | 5 | 0.0943 | 0.0173 | | | | |
| | 2+3 | 0.2083 | 0.0696 | | | 0.389 | |

The table shows that sufficient data points were collected to demonstrate a significant ($p < 0.05$) reduction in TAN across the CCMB units for every media/trial combination. Parameters with insignificant comparison of means results should undergo additional scrutiny before using the change measured across the CCMB units (influent versus effluent) to describe or compare performance. These insignificant data would be better utilized as averages describing the conditions present at each media/trial combination. An explanation of the Trial 2 and Trial 3 grouping is explained in Section 4.1.4. The trial grouping is provided in Table 4.1.2-1 to demonstrate that the grouping did not introduce enough variability to make the already significant individual Trial 2 and 3 comparisons insignificant.

4.1.3 Trial Similarity

In order to allow meaningful comparisons between systems and trials, conditions including D.O., temperature, pH, alkalinity, and TAN were intended to remain constant or within certain limits between the three systems and during all trials. Although constant $NO_2^-$-N concentrations were not required to compare nitritation rates, results are presented for later discussion of second stage nitrification or nitratation rates.

4.1.3.1 Dissolved Oxygen

Throughout this study CCMB, average influent $O_2$ concentrations were maintained above the critical 3.54 mg/L level identified in Section 2.5.1. Although some individual influent $O_2$ concentrations did drop slightly below 3.54 mg/L (primarily during Trial 2) none of these concentrations were rate limiting when the corresponding TAN concentrations were compared against the $O_2$/TAN limiting curve shown on Figure 2.5.1-1. Therefore, according to Equation 2.5.1-1, $O_2$ was maintained at high enough concentrations to assume that it was not the limiting factor for nitrification.

Table 4.1.3.1-1. Comparison of System $O_2$ Concentrations (mg/L) by Trial.

| Trial | Media | DO | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 4.67 | 5 | 0.376 | A | 0.71 | 0.5116 |
| | 2 | 4.89 | 5 | 0.326 | A | | |
| | 3 | 4.94 | 5 | 0.429 | A | | |
| 2 | 1 | 3.86 | 5 | 0.552 | A | 0.46 | 0.6412 |
| | 2 | 3.78 | 5 | 0.324 | A | | |
| | 3 | 3.55 | 5 | 0.629 | A | | |
| 3 | 1 | 4.24 | 5 | 0.214 | A | 3.00 | 0.0878 |
| | 2 | 4.61 | 5 | 0.273 | A | | |
| | 3 | 4.27 | 5 | 0.295 | A | | |
| 4 | 1 | 5.29 | 6 | 0.300 | A | 0.51 | 0.6091 |
| | 2 | 5.47 | 6 | 0.276 | A | | |
| | 3 | 5.33 | 6 | 0.361 | A | | |
| 5 | 1 | 5.22 | 6 | 0.395 | A | 6.50 | 0.0093 |
| | 2 | 5.52 | 6 | 0.285 | AB | | |
| | 3 | 5.88 | 6 | 0.246 | B | | |

Table 4.1.3.1-1 shows that for Trials 1 through 4 there were no significant ($p < 0.05$) differences in average influent $O_2$ concentrations between systems. Trial 5 did have a significant ($p > 0.05$) difference primarily between Systems 1 and 3 (see Tukey Grouping), although at concentrations above 5 mg/L (>>3.54 mg/L) it is assumed that the differences observed had little impact on system performance.

Table 4.1.3.2-2. Comparison of Trial $O_2$ Concentrations (mg/L) by System.

| Media | Trial | Temp | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 4.67 | 5 | 0.376 | BC | 14.34 | <0.0001 |
| | 2 | 3.86 | 5 | 0.552 | A | | |
| | 3 | 4.24 | 5 | 0.214 | AB | | |
| | 4 | 5.29 | 6 | 0.300 | C | | |
| | 5 | 5.22 | 6 | 0.395 | C | | |
| 2 | 1 | 4.89 | 5 | 0.326 | B | 31.06 | <0.0001 |
| | 2 | 3.78 | 5 | 0.324 | A | | |
| | 3 | 4.61 | 5 | 0.273 | B | | |
| | 4 | 5.47 | 6 | 0.276 | C | | |
| | 5 | 5.52 | 6 | 0.285 | C | | |
| 3 | 1 | 4.94 | 5 | 0.429 | BC | 27.03 | <0.0001 |
| | 2 | 3.55 | 5 | 0.629 | A | | |
| | 3 | 4.27 | 5 | 0.295 | AB | | |
| | 4 | 5.33 | 6 | 0.361 | CD | | |
| | 5 | 5.88 | 6 | 0.246 | D | | |

In comparing average influent $O_2$ concentrations across trials, Table 4.1.3.2-2 shows significant ($p > 0.05$) differences for each system. In each case there was a significant reduction in $O_2$ between Trials 1 and 2 followed by increases for Trials 3 and 4. These $O_2$ shifts were caused by changes in the performance of the air supply system. After the observed reduction in $O_2$ during Trial 2, it was discovered that both the air intake filter on the air supply blower required changing and the ventilation fan for the blower room had failed. A fresh filter and restored blower room ventilation resulted in

the increase recorded during Trial 3. Subsequent increases in $O_2$ resulted when further actions were taken to reduce the temperature in the blower room.

As previously discussed, none of the $O_2$ concentrations observed were rate limiting. Therefore, changes in $O_2$ across trials should have little impact on system performance.

4.1.3.2 Temperature

Temperatures throughout this study were maintained at an average of 26 °C with a standard deviation of 0.67. Though this constancy was better than anticipated, the differences measured warrant discussion.

Table 4.1.3.2-1. Comparison of System Temperatures (°C) by Trial.

| Trial | Media | Temp | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 25.2 | 5 | 0.964 | A | 1.21 | 0.3320 |
| | 2 | 25.4 | 5 | 0.819 | A | | |
| | 3 | 24.5 | 5 | 1.018 | A | | |
| 2 | 1 | 25.7 | 5 | 0.610 | A | 2.50 | 0.1238 |
| | 2 | 25.9 | 5 | 0.471 | A | | |
| | 3 | 25.2 | 5 | 0.485 | A | | |
| 3 | 1 | 26.6 | 5 | 0.554 | A | 3.36 | 0.0695 |
| | 2 | 26.8 | 5 | 0.513 | A | | |
| | 3 | 25.8 | 5 | 0.736 | A | | |
| 4 | 1 | 26.6 | 6 | 0.098 | A | 34.63 | <0.0001 |
| | 2 | 26.5 | 6 | 0.288 | A | | |
| | 3 | 25.8 | 6 | 0.041 | B | | |
| 5 | 1 | 26.7 | 6 | 0.075 | A | 50.61 | <0.0001 |
| | 2 | 26.6 | 6 | 0.105 | A | | |
| | 3 | 26.1 | 6 | 0.147 | B | | |

Table 4.1.3.2-1 shows significant ($p < 0.05$) differences between system temperatures during Trials 4 and 5 and nearly so for Trial 3. It is easily observed that for all trials, the temperature of System 3 was lower than that of the other two systems. The difference between the temperature of System 3 (the coolest system) and the

temperature of the warmest system ranged from a minimum of 0.6 °C during Trial 5 to a maximum of 0.9 °C during Trial 3 with an average maximum difference of 0.8 °C.

Differences in temperature were also compared for each system individually over the duration of all trials. Table 4.1.3.2-2 shows significant ($p < 0.05$) differences between trial temperatures for all systems. Maximum temperature differences between trials on each of the individual systems were very close to one another and averaged 1.5 °C. The general trend was an increase in temperature from Trial 1 to Trial 5, primarily due to the warming of the season and a gradual failure of the building's climate control system (central air conditioning) that had been relied upon to regulate the temperature of the experimental systems.

Table 4.1.3.2-2. Comparison of trial temperatures (°C) by system.

| Media | Trial | Temp | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 25.2 | 5 | 0.964 | A | 8.04 | 0.0004 |
| | 2 | 25.7 | 5 | 0.610 | AB | | |
| | 3 | 26.6 | 5 | 0.554 | BC | | |
| | 4 | 26.6 | 6 | 0.098 | BC | | |
| | 5 | 26.7 | 6 | 0.075 | C | | |
| 2 | 1 | 25.4 | 5 | 0.819 | A | 6.78 | 0.0010 |
| | 2 | 25.9 | 5 | 0.471 | A | | |
| | 3 | 26.8 | 5 | 0.513 | B | | |
| | 4 | 26.5 | 6 | 0.288 | B | | |
| | 5 | 26.6 | 6 | 0.105 | B | | |
| 3 | 1 | 24.5 | 5 | 1.018 | A | 6.26 | 0.0016 |
| | 2 | 25.2 | 5 | 0.485 | A | | |
| | 3 | 25.8 | 5 | 0.736 | B | | |
| | 4 | 25.8 | 6 | 0.041 | B | | |
| | 5 | 26.1 | 6 | 0.147 | B | | |

Respective differences of 0.8 and 1.5 °C for the average maximum temperature difference between systems in the same trial and for trials of the same system, while

statistically significant, are impossible to definitively evaluate against filter performance without a temperature vs. performance study unique to this type of system. Also, the differences are not dissimilar enough to warrant modifying the recorded results using temperature-performance relationships published for other systems. System 3 did have the lower temperature and does appear to have the poorest nitritation performance (Section 4.2), however the use of different media and other problems plaguing System 3 preclude temperature from being seriously considered as the cause of the lower nitrification rates. Therefore, it was decided that the temperature differences measured throughout this study would not be used to show differences between system or trial environments.

DeLosReyes (1995) also observed a significant temperature difference (2.6 °C) between two systems operated at ambient conditions in the same physical location as this research but did not address it's impact on system performance. In fact, no research reviewed during an intensive literature review specifically addressed the effect of small consistent temperature differences between experimental units on filter performance.

4.1.3.3 pH

A comparison of recorded pH observations equivalent to those of the previously discussed parameters is impossible as pH was only measured once per day during the two 24-hour trials (Trials 4 and 5). However, Table 4.1.3.3-1 does show significant ($p < 0.05$) pH differences between System 2 and Systems 1 and 3 during Trials 2 and 3.

Because all pH values recorded were near or above the target pH of 7.5, it is assumed that the observed changes in pH had little impact on system performance.

Table 4.1.3.3-1. Comparison of system pH by trial.

| Trial | Media | pH | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 7.47 | 5 | 0.070 | A | 0.17 | 0.8416 |
| | 2 | 7.50 | 5 | 0.055 | A | | |
| | 3 | 7.48 | 5 | 0.051 | A | | |
| 2 | 1 | 7.47 | 5 | 0.022 | A | 14.53 | 0.0006 |
| | 2 | 7.52 | 5 | 0.015 | B | | |
| | 3 | 7.47 | 5 | 0.013 | A | | |
| 3 | 1 | 7.49 | 5 | 0.027 | A | 5.34 | 0.0219 |
| | 2 | 7.52 | 5 | 0.025 | A | | |
| | 3 | 7.47 | 5 | 0.028 | A | | |
| 4 | 1 | 7.58 | 1 | * | * | * | * |
| | 2 | 7.61 | 1 | * | * | | |
| | 3 | 7.59 | 1 | * | * | | |
| 5 | 1 | 7.67 | 1 | * | * | * | * |
| | 2 | 7.69 | 1 | * | * | | |
| | 3 | 7.73 | 1 | * | * | | |

* pH was measured only once during the 24-hour diurnal trials.

Table 4.1.3.3-2. Comparison of Trial pH by System.

| Media | Trial | pH | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 7.47 | 5 | 0.070 | A | 5.39 | 0.0101 |
| | 2 | 7.47 | 5 | 0.022 | A | | |
| | 3 | 7.49 | 5 | 0.027 | A | | |
| | 4 | 7.58 | 1 | * | AB | | |
| | 5 | 7.67 | 1 | * | B | | |
| 2 | 1 | 7.50 | 5 | 0.055 | A | 7.38 | 0.0031 |
| | 2 | 7.52 | 5 | 0.015 | A | | |
| | 3 | 7.52 | 5 | 0.025 | A | | |
| | 4 | 7.61 | 1 | * | AB | | |
| | 5 | 7.69 | 1 | * | B | | |
| 3 | 1 | 7.48 | 5 | 0.051 | AB | 15.01 | 0.0001 |
| | 2 | 7.47 | 5 | 0.013 | A | | |
| | 3 | 7.47 | 5 | 0.028 | AB | | |
| | 4 | 7.59 | 1 | * | BC | | |
| | 5 | 7.73 | 1 | * | C | | |

* pH was measured only once during the 24-hour diurnal trials.

A comparison of pH for each system individually over the duration of all trials can be made and is shown in Table 4.1.3.3-2 to have had significant ($p < 0.05$) differences

between pH values recorded during Trials 1, 2, and 3 versus Trial 5 for all Systems and between Trial 2 versus Trials 4 and 5 for System 3.

Again, because all pH values recorded were near or above the target pH of 7.5, it is assumed that the observed changes in pH had little impact on system performance.

4.1.3.4 Alkalinity

As with pH and discussed in the previous subsection, alkalinity was only measured once per day during the two 24-hour trials (Trials 4 and 5). Alkalinity was slightly more constant than pH with significant ($p < 0.05$) Alkalinity differences between System 2 and Systems 1 and 3 during only Trial 2 as illustrated in Table 4.1.3.4-1.

Because all alkalinity values recorded were greater than the target alkalinity of 150 mg/L as $CaCO_3$, it is assumed that the observed changes in alkalinity had little impact on system performance.

Table 4.1.3.4-1. Comparison of System Alkalinity (mg/L as $CaCO_3$) by Trial.

| Trial | Media | Alk | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 161.40 | 5 | 12.621 | A | 0.43 | 0.6618 |
| | 2 | 155.80 | 5 | 6.099 | A | | |
| | 3 | 157.80 | 5 | 9.284 | A | | |
| 2 | 1 | 163.20 | 5 | 5.167 | A | 5.19 | 0.0237 |
| | 2 | 172.40 | 5 | 2.408 | B | | |
| | 3 | 169.00 | 5 | 5.477 | AB | | |
| 3 | 1 | 163.00 | 5 | 2.739 | A | 0.08 | 0.9212 |
| | 2 | 163.00 | 5 | 5.612 | A | | |
| | 3 | 162.00 | 5 | 4.637 | A | | |
| 4 | 1 | 163.00 | 1 | * | | | |
| | 2 | 166.00 | 1 | * | | | |
| | 3 | 170.00 | 1 | * | | | |
| 5 | 1 | 177.00 | 1 | * | | | |
| | 2 | 177.00 | 1 | * | | | |
| | 3 | 177.00 | 1 | * | | | |

* Alk. was measured only once during the 24-hour diurnal trials.

Alkalinity again proves to be more constant than pH when comparing each system individually over the duration of all trials. Table 4.1.3.4-2 shows significant ($p < 0.05$) differences between alkalinity values recorded during Trial 1 versus Trials 2 and 5 only within System 2.

Again, because all alkalinity values recorded were greater than the target alkalinity of 150 mg/L as $CaCO_3$, it is assumed that the observed changes in alkalinity had little impact on system performance.

Table 4.1.3.4-2. Comparison of Trial Alkalinity (mg/L as $CaCO_3$) by System.

| Media | Trial | Alk | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 161.40 | 5 | 12.621 | A | 0.80 | 0.5486 |
| | 2 | 163.20 | 5 | 5.167 | A | | |
| | 3 | 163.00 | 5 | 2.739 | A | | |
| | 4 | 163.00 | 1 | * | A | | |
| | 5 | 177.00 | 1 | * | A | | |
| 2 | 1 | 155.80 | 5 | 6.099 | A | 8.66 | 0.0016 |
| | 2 | 172.40 | 5 | 2.408 | B | | |
| | 3 | 163.00 | 5 | 5.612 | AB | | |
| | 4 | 166.00 | 1 | * | AB | | |
| | 5 | 177.00 | 1 | * | B | | |
| 3 | 1 | 157.80 | 5 | 9.284 | A | 2.95 | 0.0653 |
| | 2 | 169.00 | 5 | 5.477 | A | | |
| | 3 | 162.00 | 5 | 4.637 | A | | |
| | 4 | 170.00 | 1 | * | A | | |
| | 5 | 177.00 | 1 | * | A | | |

* Alk. was measured only once during the 24-hour diurnal trials.

4.1.3.5 Total Ammonia Nitrogen

Influent TAN (TANin) concentrations were maintained below the recommended 1 mg/L (Lawson, 1995; Malone and DeLosReyes, 1997) and there were no significant ($p < 0.05$) differences between systems or trials as indicated in Tables 4.1.3.5-1 and 4.1.3.5-2. Also, as nitrification is generally linear at nitrogen substrate concentrations

less than 1 mg/L (Iwai and Kitao, 1994), the differences observed can be easily normalized

Table 4.1.3.5-1. Comparison of System TANin (mg/L) Concentrations by Trial.

| Trial | Media | TANin | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 0.74 | 5 | 0.198 | A | 0.2 | 0.8244 |
| | 2 | 0.67 | 5 | 0.192 | A | | |
| | 3 | 0.77 | 5 | 0.335 | A | | |
| 2 | 1 | 0.64 | 5 | 0.359 | A | 0.11 | 0.8995 |
| | 2 | 0.66 | 5 | 0.395 | A | | |
| | 3 | 0.75 | 5 | 0.449 | A | | |
| 3 | 1 | 0.87 | 5 | 0.090 | A | 1.24 | 0.3247 |
| | 2 | 0.81 | 5 | 0.093 | A | | |
| | 3 | 0.90 | 5 | 0.087 | A | | |
| 4 | 1 | 0.64 | 6 | 0.171 | A | 0.22 | 0.8073 |
| | 2 | 0.70 | 6 | 0.154 | A | | |
| | 3 | 0.64 | 6 | 0.193 | A | | |
| 5 | 1 | 0.61 | 6 | 0.208 | A | 2.69 | 0.1002 |
| | 2 | 0.80 | 6 | 0.296 | A | | |
| | 3 | 0.50 | 6 | 0.158 | A | | |

Table 4.1.3.5-2. Comparison of Trial TANin (mg/L) Concentrations by System.

| Media | Trial | TANin | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 0.74 | 5 | 0.198 | A | 1.23 | 0.3277 |
| | 2 | 0.64 | 5 | 0.359 | A | | |
| | 3 | 0.87 | 5 | 0.090 | A | | |
| | 4 | 0.64 | 6 | 0.171 | A | | |
| | 5 | 0.61 | 6 | 0.208 | A | | |
| 2 | 1 | 0.67 | 5 | 0.192 | A | 0.45 | 0.7744 |
| | 2 | 0.66 | 5 | 0.395 | A | | |
| | 3 | 0.81 | 5 | 0.093 | A | | |
| | 4 | 0.70 | 6 | 0.154 | A | | |
| | 5 | 0.80 | 6 | 0.296 | A | | |
| 3 | 1 | 0.77 | 5 | 0.335 | A | 1.69 | 0.1872 |
| | 2 | 0.75 | 5 | 0.449 | A | | |
| | 3 | 0.90 | 5 | 0.087 | A | | |
| | 4 | 0.64 | 6 | 0.193 | A | | |
| | 5 | 0.50 | 6 | 0.158 | A | | |

4.1.3.6 Nitrite

CCMB influent $NO_2^-$-N ($NO_2^-$-Nin) concentrations were maintained below the recommended 1 mg/L concentration for average aquaculture systems (Malone and DeLosReyes, 1997) for all trials except Trials 2 and 3 in the Media 3 system. The cause of the elevated $NO_2^-$-N concentrations was most likely attributed to the clogged pipes and associated anaerobic conditions described in Section 4.8, as the concentrations did reduce to levels lower than recorded in the other two systems after the problem was corrected. Differences between media systems and trials as indicated in Tables 4.1.3.6-1 and 4.1.3.6-2 were normalized in calculating nitratation rates as described in Section 2.9, despite the one large outlier of 3.51 mg/L recorded for the Media 3 system during trial 3.

Table 4.1.3.6-1. Comparison of System $NO_2^-$-Nin (mg/L) Concentrations by Trial.

| Trial | Media | $NO_2^-$-Nin | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 0.06 | 5 | 0.016 | A | 6.88 | 0.0102 |
| | 2 | 0.11 | 5 | 0.025 | B | | |
| | 3 | 0.08 | 5 | 0.021 | AB | | |
| 2 | 1 | 0.51 | 5 | 0.398 | A | 6.10 | 0.0149 |
| | 2 | 0.64 | 5 | 0.306 | AB | | |
| | 3 | 1.18 | 5 | 0.249 | B | | |
| 3 | 1 | 0.44 | 5 | 0.052 | A | 498.20 | <0.0001 |
| | 2 | 0.61 | 5 | 0.037 | A | | |
| | 3 | 3.51 | 5 | 0.293 | B | | |
| 4 | 1 | 0.93 | 6 | 0.131 | A | 8.18 | 0.0040 |
| | 2 | 0.60 | 6 | 0.223 | B | | |
| | 3 | 0.92 | 6 | 0.105 | A | | |
| 5 | 1 | 0.47 | 6 | 0.094 | AB | 3.69 | 0.0497 |
| | 2 | 0.52 | 6 | 0.086 | A | | |
| | 3 | 0.39 | 6 | 0.061 | B | | |

Table 4.1.3.6-2. Comparison of Trial $NO_2^-$-Nin (mg/L) Concentrations by media.

| Media | Trial | NO2-Nin | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 0.06 | 5 | 0.016 | A | 14.92 | <0.0001 |
| | 2 | 0.51 | 5 | 0.398 | B | | |
| | 3 | 0.44 | 5 | 0.052 | BC | | |
| | 4 | 0.93 | 6 | 0.131 | D | | |
| | 5 | 0.47 | 6 | 0.094 | BC | | |
| 2 | 1 | 0.11 | 5 | 0.025 | A | 8.14 | 0.0003 |
| | 2 | 0.64 | 5 | 0.306 | B | | |
| | 3 | 0.61 | 5 | 0.037 | B | | |
| | 4 | 0.60 | 6 | 0.223 | B | | |
| | 5 | 0.52 | 6 | 0.086 | B | | |
| 3 | 1 | 0.08 | 5 | 0.021 | A | 308.77 | <0.0001 |
| | 2 | 1.18 | 5 | 0.249 | B | | |
| | 3 | 3.51 | 5 | 0.293 | C | | |
| | 4 | 0.92 | 6 | 0.105 | B | | |
| | 5 | 0.39 | 6 | 0.061 | A | | |

4.1.4 Organic Loading

Before the effect of organic loading on biofilter performance could be evaluated, the organic loading results had to be screened to ensure the objectives could be met. As described in Section 4.1.2, for most data there was not a statistically significant difference between the CCMB influent and effluent concentrations. Therefore, both influent and effluent data were combined for use in defining system conditions (organic loading regime). Table 4.1.4-1 shows the comparison between the COD data sets for all trials by media.

Of the five trials conducted, Trials 2, 3, and 4 were designed to provide 3 successively increasing organic loading rates by removing components of the solids-removal device after each trial while keeping all other operating conditions constant. Trial 2 utilized a settling basin with tube-settler media; in Trial 3 the settling basin was used without the tube-settler media; and in Trial 4 the settling basin was completely

bypassed. However, a specific comparison of the COD (influent and effluent) values measured during Trials 2, 3, and 4 (see Table 4.1.4-2) showed that only Trial 4 had a significantly different COD level and that there were virtually no differences between the COD levels recorded during Trials 2 and 3.

Table 4.1.4-1. Comparison of mean COD (mg/L) concentrations of trials by media system.

| Media | Trial | Mean COD | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 21.90 | 5 | 2.104 | A | 776.88 | <0.0001 |
| | 2 | 94.40 | 5 | 8.287 | B | | |
| | 3 | 104.90 | 5 | 7.545 | B | | |
| | 4 | 147.08 | 6 | 4.873 | C | | |
| | 5 | 205.25 | 6 | 3.818 | D | | |
| 2 | 1 | 21.90 | 5 | 5.482 | A | 684.45 | <0.0001 |
| | 2 | 83.70 | 5 | 6.970 | B | | |
| | 3 | 82.90 | 5 | 4.574 | B | | |
| | 4 | 153.33 | 6 | 6.882 | C | | |
| | 5 | 197.50 | 6 | 6.197 | D | | |
| 3 | 1 | 13.50 | 5 | 3.758 | A | 282.27 | <0.0001 |
| | 2 | 90.20 | 5 | 11.595 | B | | |
| | 3 | 87.20 | 5 | 7.059 | B | | |
| | 4 | 145.58 | 6 | 9.292 | C | | |
| | 5 | 172.92 | 6 | 8.645 | D | | |

Table 4.1.4-2. Comparison of Trials 2, 3, and 4 mean COD (mg/L) concentrations by media system.

| Media | Trial | Mean COD | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 1 | 2 | 94.40 | 5 | 8.29 | A | 91.18 | <0.0001 |
| | 3 | 104.90 | 5 | 7.54 | A | | |
| | 4 | 147.08 | 6 | 4.87 | B | | |
| 2 | 2 | 83.70 | 5 | 6.97 | A | 232.23 | <0.0001 |
| | 3 | 82.90 | 5 | 4.57 | A | | |
| | 4 | 153.30 | 6 | 6.88 | B | | |
| 3 | 2 | 90.20 | 5 | 11.6 | A | 67.60 | <0.0001 |
| | 3 | 87.20 | 5 | 7.06 | A | | |
| | 4 | 145.58 | 6 | 9.29 | B | | |

In order to show the impact of organic loading on CCMB nitrification performance, Trials 2 and 3 with their similar COD levels were grouped together and compared against Trial 4. A comparison of the COD values measured during Trials 2, 3, and 4 is presented in Table 4.1.4-3 with the new Trial grouping and shows a much improved definition of the difference between the COD levels during Trial 4 and the combination of Trials 2 and 3.

Table 4.1.4-3. Comparison of Trials 2 and 3 combined and Trial 4 mean COD (mg/L) concentrations by media system.

| Media | Trial | Mean COD | N | Std Dev | T | p |
|---|---|---|---|---|---|---|
| 1 | 2+3 | 99.65 | 10 | 9.30 | 11.48 | <0.0001 |
| | 4 | 147.08 | 6 | 4.87 | | |
| 2 | 2+3 | 83.30 | 10 | 5.57 | 22.33 | <0.0001 |
| | 4 | 153.30 | 6 | 6.88 | | |
| 3 | 2+3 | 88.70 | 10 | 9.19 | 11.94 | <0.0001 |
| | 4 | 145.58 | 6 | 9.29 | | |

Table 4.1.4-4. Comparison of media system mean COD (mg/L) concentrations by trial grouping.

| Trial | Media | Mean COD | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 2+3 | 1 | 99.65 | 10 | 9.30 | A | 10.31 | 0.0005 |
| | 2 | 83.30 | 10 | 5.57 | B | | |
| | 3 | 88.70 | 10 | 9.19 | B | | |
| 4 | 1 | 147.08 | 6 | 4.87 | A | 1.93 | 0.1793 |
| | 2 | 153.30 | 6 | 6.88 | A | | |
| | 3 | 145.58 | 6 | 9.29 | A | | |

Although the mean COD concentration observed for the Trial 2 and Trial 3 grouping was significantly ($p<0.05$) greater and approximately 16% higher for the Media 1 system than for the Media 2 and Media 3 systems, a comparison presented in Table 4.1.4-4 shows the grouping works well to create two distinct COD regimes. The

mean of all media systems during the combination of Trials 2 and 3 was 91 mg/L compared to the mean COD for Trial 4 of 149 mg/L, an increase of 64%.

**4.2 Effect of Media Selection Filter Performance**

In comparing mean volumetric nitritation rates ( $C_A^{V'}$ ) against media types at each of the two COD load trial groupings (see Table 4.2-1), the performances of the three media types were ranked Media 1 > Media 2 = Media 3, with the performance of Media 1 significantly greater than the other two media types and that of Media 2 indistinguishable from Media 3.

It is coincidental that the original CCMB media would outperform the two alternate media types selected for this study, however there are a few potential explanations for this observation. Turbulence within the CCMB can affect biofilm detachment primarily via shear and particle to particle interactions. The first mechanism, shear, has more impact on larger particles than smaller ones. Table 3.1.1-2 shows that an average pellet of Media 1, 2, and 3 had a bulk volume of 2.90, 2.36, 2.57 $x10^{-3}$ $ft^3$ (82.2, 66.8, 72.7 $cm^3$), respectively. Therefore, shear could have affected Media 1 > Media 3 > Media 2. This order does not agree with the nitritation performance order, so perhaps shear affects *Nitrosomonas* detachment on CCMB media to a lesser extent than in other systems, or another variable is dominant. The second mechanism, particle to particle interactions, could be affected by particle sphericity in two ways.

1. Theoretical stress region: objects with less sphericity have a larger radius of curvature, thus creating a larger impact area to dissipate and minimize contact stresses from collisions.

2. Physical packing geometry: a visual observation made during this study was that Media 2 appeared to be the most spherical and did not "lock" together in the annular or "packed bed" region of the CCMB (see Figures 3.1.1-1 and 3.1.1-4). Rather, the media particles seemed to continuously shift and grind against one another as the packed bed moved up the annulus of the CCMB.

Table 3.1.1-2 shows that average pellets of Media 1, 2, and 3 had respective sphericities of 83.7%, 86.7%, and 82.3%. Therefore, particle to particle interactions could possibly have affected Media 2 greater than Media 1 or Media 3, which were about equal in sphericity. This order also does not agree with the exact nitritation performance order, but does at least suggest that the top performing Media 1 might be less impacted by biofilm abrasion losses than the poorer performing Media 2.

Table 4.2-1. Comparison of volumetric nitritation rates ($C_A^{V'}$) normalized for influent TAN as g TAN/day-$m^3$ (lb TAN/day-$ft^3$) of media systems by COD load trial grouping.

| Trial | Media | Mean $C_A^{V'}$ | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 2+3 | 1 | 237 (0.0148) | 10 | 26 (0.0016) | A | 32.29 | <0.0001 |
| | 2 | 175 (0.0109) | 10 | 14 (0.0008) | B | | |
| | 3 | 167 (0.0105) | 10 | 23 (0.0014) | B | | |
| 4 | 1 | 217 (0.0135) | 6 | 47 (0.0030) | A | 3.99 | 0.0409 |
| | 2 | 162 (0.0101) | 6 | 21 (0.0013) | A | | |
| | 3 | 160 (0.0100) | 6 | 45 (0.0028) | A | | |

A possible explanation of the poorer performance of Media 3 could have been its apparent persistent hydrophobic nature. All plastic media types encountered have been hydrophobic when first placed in the CCMB. However, Media 3 remained extremely hydrophobic until almost a week into the acclimation phase and somewhat hydrophobic throughout the entire study. The other two media types lost all hydrophobic

characteristics after just a few days into the shakedown period, before acclimation began.

As mentioned in Section 2.4, it is generally assumed that nitritation is the limiting step of the two-step nitrification process and so nitratation performance is seldom evaluated in aquaculture biofilter research. Unfortunately, this assumption could not be blindly adopted because $NO_2^-$-N concentrations exceeded 1 mg/L during Trials 2 and 3 of System 3. Also, culture tank, equal to CCMB influent, $NO_2^-$-N concentrations were greater than TAN concentrations during Trials 2 and 4 of System 1, Trial 2 of System 2, and Trails 2, 3, and 4 of System 3. Evaluating nitratation performance could offer support that these conditions were the result of $NO_2^-$-N production by denitrifying bacteria somewhere else in the systems.

Table 4.2-2. Comparison of volumetric nitratation rates ($C_N^{V'}$) normalized for influent TAN and $NO_2^-$-N as g $NO_2^-$-N/day-m$^3$ (lb $NO_2^-$-N/day-ft$^3$) of media systems by COD load trial grouping.

| Trial | Media | Mean $C_N^{V'}$ | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 2+3 | 1 | 313 (0.0195) | 10 | 108 (0.0068) | A | 30.29 | <0.0001 |
| | 2 | 172 (0.0107) | 10 | 36 (0.0022) | B | | |
| | 3 | 188 (0.0117) | 10 | 38 (0.0024) | B | | |
| 4 | 1 | 241 (0.0150) | 6 | 57 (0.0036) | A | 3.59 | 0.0533 |
| | 2 | 173 (0.0108) | 6 | 40 (0.0025) | B | | |
| | 3 | 195 (0.0121) | 6 | 57 (0.0035) | AB | | |

The mean volumetric nitratation ($C_N^{V'}$) performances of the three media types were ranked similar to $C_A^{V'}$ with Media 1 > Media 3 = Media 2 for the lower COD loading trial grouping, while for the higher COD loading Media 3 = Media 1 > Media 2 = Media 3. Again, the performance of Media 1 was greater that those of the other two media types, although this difference was both significant and almost 2.4 times greater

at the lower COD loading trial grouping and not significant at the higher loading. The performance of Media 3 was not significantly greater than that of Media 2. The same arguments used to offer possible explanations for differences between media performance for $C_A^{V'}$ can also be used for $C_N^{V'}$.

Volumetric rates are important to the end-user as they determine the physical size of the biofilter in a space-limited industry. However, areal rates can help researchers evaluate the activity of the biofilm and may aid in optimizing system performance.

Table 4.2-3. Comparison of areal nitritation rates ($C_A^{A'}$) normalized for influent TAN as mg TAN/day-m$^2$ (lb TAN/day ft$^2$ x10$^{-5}$) of media systems by COD load trial grouping.

| Trial | Media | Mean $C_A^{A'}$ | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 2+3 | 1 | 217 (4.44) | 10 | 24 (0.48) | A | 51.90 | <0.0001 |
| | 2 | 151 (3.10) | 10 | 12 (0.24) | B | | |
| | 3 | 137 (2.81) | 10 | 19 (0.38) | B | | |
| 4 | 1 | 198 (4.06) | 6 | 43 (0.89) | A | 6.67 | 0.0085 |
| | 2 | 140 (2.87) | 6 | 18 (0.37) | B | | |
| | 3 | 131 (2.69) | 6 | 37 (0.76) | B | | |

Table 4.2-4. Comparison of areal nitratation rates ($C_N^{A'}$) normalized for influent TAN and $NO_2^-$-N as mg $NO_2^-$-N/day-m$^2$ (lb $NO_2^-$-N/day-ft$^2$ x10$^{-5}$) of media systems by COD load trial grouping.

| Trial | Media | Mean $C_N^{A'}$ | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 2+3 | 1 | 286 (5.86) | 10 | 99 (2.03) | A | 37.79 | <0.0001 |
| | 2 | 149 (3.05) | 10 | 31 (0.63) | B | | |
| | 3 | 154 (3.16) | 10 | 31 (0.64) | B | | |
| 4 | 1 | 220 (4.51) | 6 | 52 (1.07) | A | 5.71 | 0.0144 |
| | 2 | 150 (3.07) | 6 | 35 (0.71) | B | | |
| | 3 | 160 (3.27) | 6 | 47 (0.95) | B | | |

The statistical results from comparing the nitrification rates of media systems by COD load trial grouping showed more significant differences for the areal data than the volumetric data. The only reason for this difference was due to the differences in the media surface area to volume ratios and their incorporation into the statistical results. Areal nitritation and nitratation comparisons of media systems by COD load trial grouping are shown in Table 4.2-3 and Table 4.2-4, respectively.

**4.3 Effect of Organic Loading on Filter Performance**

Mean volumetric nitritation rates ( $C_A^{V'}$ ) were calculated and normalized for CCMB influent TAN concentrations as described in Section 2.9 and for each media system compared against the two COD load trial groupings (see Table 4.3-1). For each media type the mean $C_A^{V'}$ not significantly ($p>0.05$) greater at the lower COD loading than at the higher loading. These observations seem to agree with the discussion presented in Section 2.5.7 that heterotrophs are capable of greater competition with nitrifiers as organic loading increases, but with the effect being less pronounced for thin biofilm systems.

Table 4.3-1. Comparison of volumetric nitritation rates ( $C_A^{V'}$ ) normalized for influent TAN as g TAN/day-$m^3$ (lb TAN/day-$ft^3$) of COD load trial groupings by media system.

| Media | Trial | Mean $C_A^{V'}$ | N | Std Dev | T | p |
|---|---|---|---|---|---|---|
| 1 | 2+3 | 237 (0.0148) | 10 | 26 (0.0016) | 1.13 | 0.2763 |
| | 4 | 217 (0.0135) | 6 | 47 (0.0030) | | |
| 2 | 2+3 | 175 (0.0109) | 10 | 14 (0.0008) | 1.54 | 0.1463 |
| | 4 | 162 (0.0101) | 6 | 21 (0.0013) | | |
| 3 | 2+3 | 167 (0.0105) | 10 | 23 (0.0014) | 0.46 | 0.6568 |
| | 4 | 160 (0.0100) | 6 | 45 (0.0028) | | |

Table 4.3-2. Comparison of volumetric nitratation rates ( $C_N^{V'}$ ) normalized for influent TAN and $NO_2^-$-N as g $NO_2^-$-N/day-$m^3$ (lb $NO_2^-$-N/day-$ft^3$) of COD load trial groupings by media system.

| Media | Trial | Mean $C_N^{V'}$ | N | Std Dev | Tukey Grouping | T | p |
|---|---|---|---|---|---|---|---|
| 1 | 2+3 | 313 (0.0195) | 10 | 108 (0.0068) | A | 2.16 | 0.0485 |
| | 4 | 241 (0.0150) | 6 | 57 (0.0036) | B | | |
| 2 | 2+3 | 172 (0.0107) | 10 | 36 (0.0022) | A | -0.10 | 0.9324 |
| | 4 | 173 (0.0108) | 6 | 40 (0.0025) | A | | |
| 3 | 2+3 | 188 (0.0117) | 10 | 38 (0.0024) | A | -0.36 | 0.7211 |
| | 4 | 195 (0.0121) | 6 | 57 (0.0035) | A | | |

The mean volumetric nitratation rates ( $C_N^{V'}$ ) shown in Table 4.3-2 were calculated to account for the conversion of both $NO_2^-$-N in the CCMB influent and $NO_2^-$-N generation within the CCMB as TAN is consumed through nitritation (See Equation 2.9-4). The mean $C_N^{V'}$ when compared against the two COD load trial groupings for each media system were significantly ($p<0.05$) greater, albeit slightly, at the lower COD loading than at the higher loading for Media 1, equal for Media 2, and not significantly ($p>0.05$) lower for Media 3. The slight increase in nitratation from the lower COD load to the higher for Media 3 could be attributed to underestimating nitratation rates for that system during Trials 2 and 3 from normalizing to $NO_2^-$-N concentrations in excess of 1 mg/L.

Table 4.3-3. Comparison of areal nitritation rates ( $C_A^{A'}$ ) normalized for influent TAN as mg TAN/day-$m^2$ (lb TAN/day-$ft^2$ x10-5) of COD load trial groupings by media system.

| Media | Trial | Mean $C_A^{A'}$ | N | Std Dev | T | p |
|---|---|---|---|---|---|---|
| 1 | 2+3 | 217 (4.44) | 10 | 24 (0.48) | 1.13 | 0.2763 |
| | 4 | 198 (4.06) | 6 | 43 (0.89) | | |
| 2 | 2+3 | 151 (3.10) | 10 | 12 (0.24) | 1.54 | 0.1463 |
| | 4 | 140 (2.87) | 6 | 18 (0.37) | | |
| 3 | 2+3 | 137 (2.81) | 10 | 19 (0.38) | 0.46 | 0.6568 |
| | 4 | 131 (2.69) | 6 | 37 (0.76) | | |

Table 4.3-4. Comparison of areal nitratation rates ( $C_N^{A'}$ ) normalized for influent TAN and $NO_2^-$-N as mg $NO_2^-$-N/day-$m^2$ (lb $NO_2^-$-N/day-$ft^2$ x10-5) of COD load trial groupings by media system.

| Media | Trial | Mean $C_N^{A'}$ | N | Std Dev | T | p |
|---|---|---|---|---|---|---|
| 1 | 2+3 | 286 (5.86) | 10 | 99 (2.03) | 2.16 | 0.0485 |
| | 4 | 220 (4.51) | 6 | 52 (1.07) | | |
| 2 | 2+3 | 149 (3.05) | 10 | 31 (0.63) | -0.10 | 0.9324 |
| | 4 | 150 (3.07) | 6 | 35 (0.71) | | |
| 3 | 2+3 | 154 (3.16) | 10 | 31 (0.64) | -0.36 | 0.7211 |
| | 4 | 160 (3.27) | 6 | 47 (0.95) | | |

As with volumetric nitrification, areal nitrification rates ( $C_A^{A'}$ and $C_N^{A'}$ ) were calculated and evaluated against both the COD load trial groupings and are presented in Table 4.2-3 and Table 4.2-4. The results of these comparisons were not dissimilar from those of the volumetric nitrification evaluations and no not warrant further discussion except to say that the statistical results of COD load trial grouping comparisons by media system were identical between areal and volumetric data sets because the only differences were shared and constant surface area to volume ratios.

### 4.4 Comparison of Nitritation against Nitratation Performance for the Organic Loading Trials and Media Types

Comparing the increase of $C_N^{A'}$ over $C_A^{A'}$ and the increase of $NO_2^-$-N over TAN as shown in Table 4.4-1, offers another method to evaluate the nitratation performances of the CCMB media types.

At both COD loadings, Media 1 shows $C_N^{A'}$ greater than $C_A^{A'}$, with the effect more pronounced at the lower COD loading. However, $NO_2^-$-Nin doubled between the lower and higher COD loadings. This indicates that in System 1, excess $NO_2^-$-N was being produced somewhere outside the CCMB through partial denitrification and/or partial

nitrification (nitritation only) and that Media 1 was less capable of processing excess $NO_2^-$-N at the higher organic loading rate than at the lower loading.

For Media 2, the lower COD loading actually shows $C_N^{A'}$ less than $C_A^{A'}$, while $C_N^{A'}$ was only slightly greater than $C_A^{A'}$ at the higher COD loading. And yet at both COD loadings, $NO_2^-$-Nin was 15% less than TANin. A possible explanation for these observations could be that the chronically poor performance of Media 2 encouraged a healthy population of nitrifiers outside of the CCMB and *in situ* nitrification provided an adequate buffer against $NO_2^-$-N accumulation. Also, System 2 never experienced any organic matter build-up or other contributors to excess $NO_2^-$-N through partial denitrification.

Finally, $C_N^{A'}$ was greater than $C_A^{A'}$ at both COD loadings for Media 3, with the difference at the lower COD loading almost half that at the higher COD loading. In contrast, the $NO_2^-$-Nin concentration at the lower COD loading was over 4 times higher than at the higher COD loading. Throughout trials 2, 3, and 4, System 3 was observed to have problems with solids collecting in the system and anaerobic partial denitrification was strongly suspected as the source of the observed $NO_2^-$-N accumulation. A particular solids accumulation problem spot in the flow distribution manifold piping to the settling basin was bypassed when the settling basins were removed from the systems after Trial 3 and explains the large drop in the steady state $NO_2^-$-N concentration between Trials 3 and 4. With the larger $NO_2^-$-N substrate concentrations at the lower COD loading, it would have been suspected that both the $C_N^{A'}$ and increase in $C_N^{A'}$ over $C_A^{A'}$ would have been greater for the lower COD loading than at the higher COD loading as observed. Perhaps the hydrophobic nature of Media

3 combined with the observations by Oga et al. (1991) that the attachment rate is lower and detachment rate higher for nitrifiers than for heterotrophs, could explain this observation. If the hydrophobic nature of Media 3 reduced the attachment effectiveness of bacteria, then the presence of more heterotrophs during the higher COD loading of Trial 4 could have given the biofilm a better hold on the media.

Table 4.4-1. Comparison of the percentage increase of nitritation over nitritation in mg $NO_2^-$-N/day-m$^2$ (lb N/day-ft$^2$ x10$^{-5}$) to the percentage increase of influent $NO_2^-$-N to TAN in mg/L of COD load trial groupings by media system.

| Media | Trial | Mean $C_A^{A'}$ | Mean $C_N^{A'}$ | Increase | TANin | $NO_2^-$-Nin | Increase |
|---|---|---|---|---|---|---|---|
| 1 | 2+3 | 217 (4.44) | 286 (5.86) | 32% | 0.75 | 0.47 | -37% |
| | 4 | 198 (4.06) | 220 (4.51) | 11% | 0.64 | 0.93 | 45% |
| 2 | 2+3 | 151 (3.10) | 149 (3.05) | -1% | 0.73 | 0.63 | -15% |
| | 4 | 140 (2.87) | 150 (3.07) | 7% | 0.70 | 0.60 | -15% |
| 3 | 2+3 | 137 (2.81) | 154 (3.16) | 12% | 0.82 | 2.35 | 185% |
| | 4 | 131 (2.69) | 160 (3.27) | 22% | 0.64 | 0.92 | 43% |

**4.5 Filter Performance for all Trials**

Although Trials 1 and 5 were out-of-scope for the objectives of this study, the statistical comparisons presented in Sections 4.2 and 4.3 were performed on all five trials and are given in Appendix C for reference and for the benefit of future research. The volumetric nitritation rates ( $C_A^{V'}$ ) of all trials are summarized by media in Table 4.5-1. Only Media 1 showed significant differences and only between Trial 1 and the other Trials. For Media 1 and Media 2, Trial 1, which had the lowest feed rate and COD concentration (see Table 4.1.4-1), also had the lowest mean $C_A^{V'}$, while Trial 1 for Media 3 had the second lowest mean $C_A^{V'}$. Perhaps the most intriguing, though not significant ($p>0.05$) observation was seen when the flow rate was increased 44% from 56.14 L/min (14.83 gpm) during Trial 4 to 80.7 L/min (21.32 gpm) during Trial 5. The

effect of flow on nitrification had been an unknown, however the results between Trial 4 and Trial 5 showed 17%, 15%, and 16% increases in mean $C_A^{V'}$ and 28%, 15%, and 17% increases in mean $C_N^{V'}$ respectively for Media types 1, 2, and 3, all while organic loading significantly increased (see Table 4.1.4-1). Originally, it had been thought that the units would have performed better at lower flow rates as the hydraulic detention time within each reactor would have been maximized. Also, the effect of increased organic loading was observed during the previously discussed organic loading trial groupings (see Section 4.3) to decrease nitrification. Though the effect of flow results were not statistically significant, it should be noted that the CCMB units were capable of even higher flow rates. Flow may have a larger effect on nitrification than organic loading and this observation may have important implications on future optimization trials.

Table 4.5-1. Comparison of volumetric nitritation rates ($C_A^{V'}$) normalized for influent TAN as g TAN/day-$m^3$ (lb TAN/day-$ft^3$) of trials by media system.

| Media | Trial | Mean $C_A^{V'}$ | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 167 (0.0105) | 5 | 43 (0.0027) | B | 4.83 | 0.0060 |
| | 2 | 228 (0.0142) | 5 | 33 (0.0021) | AB | | |
| | 3 | 247 (0.0154) | 5 | 13 (0.0008) | A | | |
| | 4 | 217 (0.0135) | 6 | 47 (0.0030) | AB | | |
| | 5 | 254 (0.0159) | 6 | 29 (0.0018) | A | | |
| 2 | 1 | 155 (0.0097) | 5 | 41 (0.0026) | A | 1.18 | 0.3454 |
| | 2 | 176 (0.0110) | 5 | 20 (0.0013) | A | | |
| | 3 | 174 (0.0109) | 5 | 3 (0.0002) | A | | |
| | 4 | 162 (0.0101) | 6 | 21 (0.0013) | A | | |
| | 5 | 187 (0.0117) | 6 | 32 (0.0020) | A | | |
| 3 | 1 | 156 (0.0098) | 5 | 47 (0.0029) | A | 1.61 | 0.2077 |
| | 2 | 148 (0.0092) | 5 | 9 (0.0006) | A | | |
| | 3 | 187 (0.0117) | 5 | 10 (0.0006) | A | | |
| | 4 | 160 (0.0100) | 6 | 45 (0.0028) | A | | |
| | 5 | 185 (0.0115) | 6 | 25 (0.0016) | A | | |

The observed significant organic loading increase between Trail 4 and Trial 5 deserves special consideration. All system operating conditions were identical between Trials 4 and 5 with the sole exception of the flow rate through the reactors. The only explanation for the increase in system COD would be that the CCMB static-zone settling described in Section 2.8.2.2 was markedly more effective at the lower flow rate of Trial 4 than at the higher flow rate of Trial 5. Taking the difference in mean COD between Trial 4 and Trial 5 and multiplying by the system volume produces the inferred COD removal rates shown in Table 4.5-2. The distinct differences in rates between systems are indirectly due to media type. In order to make the different media types recirculate properly, the ratio of flows split between the primary and secondary influent nozzles (see Figure 3.1.1-1) had to be varied. The result of this varied ratio was a variation in the internal recirculation rate between each CCMB unit. With varying internal velocities, it was not surprising that each system showed a difference in its ability to settle solids.

Table 4.5-2. Increase in system COD between Trial 4 and Trial 5 and inferred reduction in CCMB COD removal rates.

| Media/System | ÄCOD mg/L | g/day (lb/day) | kg/m3-day (lb/ft$^3$-day) |
|---|---|---|---|
| 1 | 58.2 | 225 (0.497) | 1.86 (0.1162) |
| 2 | 44.2 | 171 (0.377) | 1.41 (0.0883) |
| 3 | 27.3 | 106 (0.233) | 0.88 (0.0546) |

**4.6 Filter Acclimation**

The bacterial innoculant and chemical addition method proved effective. System acclimation periods were 16, 13, and 21 days for System 1 (Figure 4.6-1), System 2 (Figure 4.6-2), and System 3 (Figure 4.6-3), respectively. To assure an active bacterial population and prepare for stocking, $NH_4Cl$ and $NaNO_2$ were added to each system on

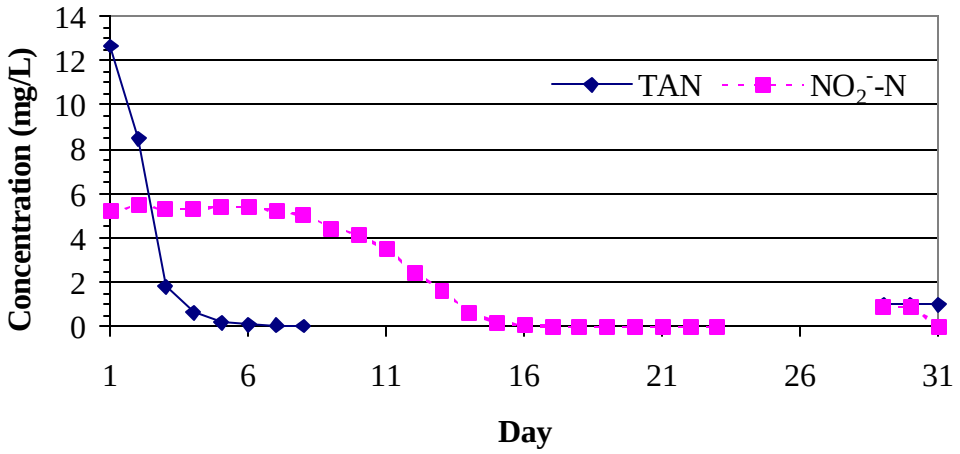


Figure 4.6-1. System 1 acclimation curve.

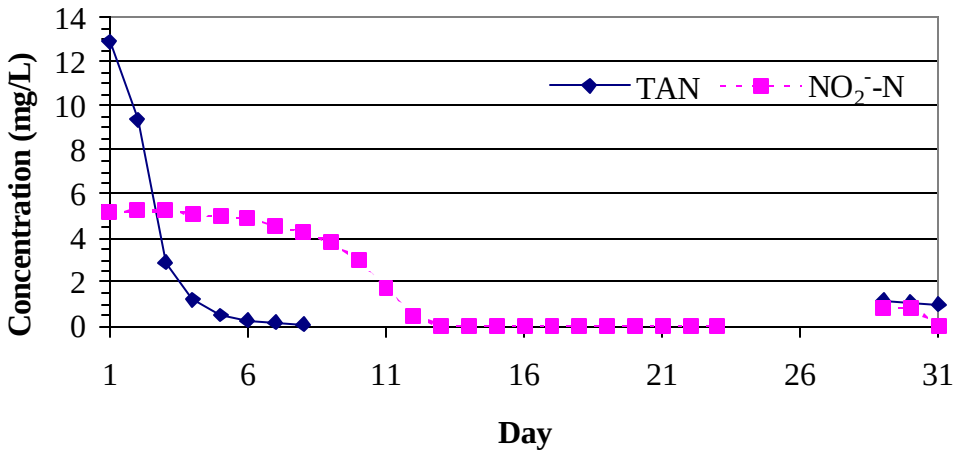


Figure 4.6-2. System 2 acclimation curve.

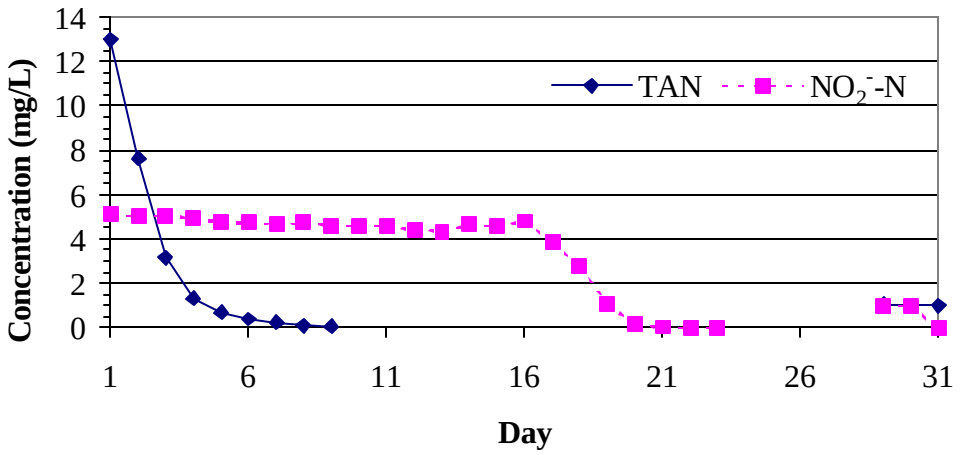


Figure 4.6-3. System 3 acclimation curve.

day 29 to increase TAN and $NO_2^-$-N levels to 1 mg/L each. On days 30 and 31 only the $NH_4Cl$ dosage was repeated. The steady TAN line for days 29 through 31 in Figure 4.1 shows that each system consumed all TAN introduced as none accumulated. The $NO_2^-$-N increased by 7.6% in System 1, 0.7% in System 2, and 1.8% in System 3 on day 30 before being depleted on day 31 and assuring acclimation.

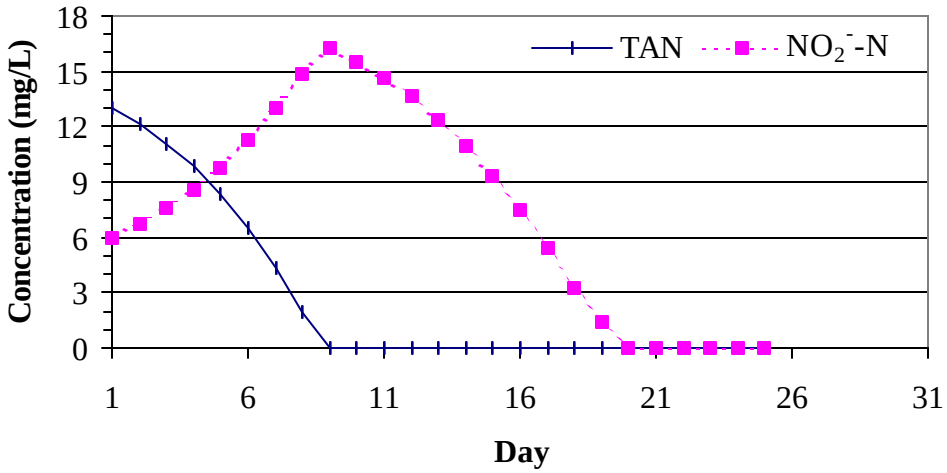


Figure 4.6-4. Theoretical acclimation curve generated by STELLA and with kinetic constants from Knowles et al. (1965).

Though the initial chemical dosing level for $NO_2^-$-N was only half of the concentration used by Manthe and Malone (1987) and DeLosReyes (1995), the acclimation results were within the 21-day referenced target. A theoretical acclimation curve (Figure 4.6-4) was generated by entering the equations presented in Section 2.5.6 into STELLA 5.0 (HPS, 1997), a visual simulation program for MS WINDOWS, with values for the kinetic constants taken from Knowles et al. (1965) and initial substrate concentrations equal to 12 and 6 mg N/L for TAN and $NO_2^-$-N, respectively. This figure differs from an acclimation curve for TAN and $NO_2^-$-N initial concentrations of 12 mg N/L each (Manthe and Malone, 1987; DeLosReyes, 1995) only because the entire $NO_2^-$-N curve has been shifted down by roughly half. By manipulating the

STELLA model it was discovered that increasing the initial biomass forced the theoretical curve to more closely resemble the acclimation curves for System 1 (Figure 4.6-1) and System 2 (Figure 4.6-2). It is quite possible that adding both commercial and active bacteria to the system introduced enough bacteria to have generated the observed results. The delay in acclimation of System 3 may be explained by the unusually strong hydrophobic nature of Media 3, as the characteristic that repelled water may have delayed the initial bacterial attachment.

**4.7 Stock Performance**

The role of tilapia in this study was solely as an organic N source for the evaluation of the CCMB units and therefore growth was not of primary concern. In fact, the tilapia chosen for these trials were larger than those normally selected for grow-out systems, primarily to avoid the early and highly exponential growth phase in the event that rapid growth would introduce additional variability between trials. However, initial and final tilapia weights and numbers were recorded and are presented in Table 4.7-1. Based on the total mass of feed provided to each system, tilapia feed conversion ratios were

Table 4.7-1. Initial and final tilapia stocking densities.

| System | Total | | Average | Stocking Density | |
|---|---|---|---|---|---|
| | Number of Fish | Weight kg (lb) | Weight kg/fish (lb/fish) | Total System kg/L (lb/gal) | Culture Tank kg/L (lb/gal) |
| Initial | | | | | |
| 1 | 200 | 42.35 (93.37) | 0.212 (0.467) | 0.00875 (0.0730) | 0.0116 (0.0972) |
| 2 | 206 | 42.45 (93.59) | 0.206 (0.454) | 0.00877 (0.0732) | 0.0117 (0.0974) |
| 3 | 205 | 42.40 (93.48) | 0.207 (0.456) | 0.00876 (0.0731) | 0.0117 (0.0973) |
| Total | 611 | 127.2 (280.4) | 0.208 (0.459) | 0.00876 (0.0731) | 0.0117 (0.0973) |
| Final | | | | | |
| 1 | 191 | 74.45 (164.1) | 0.390 (0.859) | 0.0192 (0.160) | 0.0205 (0.171) |
| 2 | 199 | 67.45 (148.4) | 0.339 (0.747) | 0.0174 (0.145) | 0.0186 (0.155) |
| 3 | 199 | 72.80 (160.5) | 0.365 (0.807) | 0.0188 (0.157) | 0.0200 (0.167) |
| Total | 589 | 221.7 (488.8) | 0.377 (0.830) | 0.0191 (0.159) | 0.0203 (0.170) |

calculated and are given in Table 4.7-2. Assuming that the tilapia did not experience significant exponential growth, linear growth rates were applied between the initial and final weights to generate stocking densities for each trial and are shown in Table 4.7-3.

Table 4.7-2. Tilapia mortalities, growth, and feed conversion ratios.

| System | Mortalities | Fish Increase kg (lb) | Feed kg (lb) | Feed Conversion Ratio unit feed/unit fish |
|---|---|---|---|---|
| 1 | 9 | 32.1 (70.8) | 95 (209) | 2.96 |
| 2 | 7 | 25.0 (55.1) | 95 (209) | 3.80 |
| 3 | 6 | 30.4 (67.0) | 95 (209) | 3.13 |
| Total | 22 | 87.5 (193) | 285 (628) | 3.26 |

Table 4.7-3. Tilapia stocking densities by trial and by system.

| Trial | System Volume L (gal) | Media | Fish kg (lb) | Stocking Density kg/L (lb/gal) | Culture Tank Stocking Density kg/L (lb/gal) |
|---|---|---|---|---|---|
| 1 | 4,840 (1,279) | 1 | 49.39 (108.9) | 0.0102 (0.0852) | 0.0136 (0.113) |
| | | 2 | 47.93 (105.7) | 0.0099 (0.0826) | 0.0132 (0.110) |
| | | 3 | 49.01 (108.0) | 0.0101 (0.0845) | 0.0135 (0.112) |
| | | Average | 48.78 (107.5) | 0.0101 (0.0841) | 0.0134 (0.112) |
| 2 | 4,840 (1,279) | 1 | 55.58 (122.5) | 0.0115 (0.0958) | 0.0153 (0.128) |
| | | 2 | 52.76 (116.3) | 0.0109 (0.0910) | 0.0145 (0.121) |
| | | 3 | 54.82 (120.9) | 0.0113 (0.0945) | 0.0151 (0.126) |
| | | Average | 54.39 (119.9) | 0.0112 (0.0938) | 0.0150 (0.125) |
| 3 | 4,840 (1,279) | 1 | 59.24 (130.6) | 0.0122 (0.1022) | 0.0163 (0.136) |
| | | 2 | 55.61 (122.6) | 0.0115 (0.0959) | 0.0153 (0.128) |
| | | 3 | 58.26 (128.4) | 0.0120 (0.1005) | 0.016 (0.134) |
| | | Average | 57.70 (127.2) | 0.0119 (0.0995) | 0.0159 (0.132) |
| 4 | 3,874 (1,023) | 1 | 64.59 (142.4) | 0.0167 (0.1391) | 0.0178 (0.148) |
| | | 2 | 59.87 (132.0) | 0.0155 (0.1290) | 0.0165 (0.137) |
| | | 3 | 63.28 (139.5) | 0.0163 (0.1363) | 0.0174 (0.145) |
| | | Average | 62.58 (138.0) | 0.0162 (0.1348) | 0.0172 (0.144) |
| 5 | 3,874 (1,023) | 1 | 71.92 (158.6) | 0.0186 (0.1549) | 0.0198 (0.165) |
| | | 2 | 65.57 (144.6) | 0.0169 (0.1412) | 0.0180 (0.150) |
| | | 3 | 70.16 (154.7) | 0.0181 (0.1511) | 0.0193 (0.161) |
| | | Average | 69.22 (152.6) | 0.0179 (0.1491) | 0.0190 (0.159) |
| Average | 4,454 (1,177) | | 58.53 (129.0) | 0.0131 (0.1097) | 0.0161 (0.134) |

### 4.8 Trial Notes

Trial 1was configured the same as Trial 2 but with a lower feed rate. This trial was not anticipated in the original scope but was performed for two reasons. First, Trial 1 served as a warm-up or test run to ensure the system, analytical methods, and fish stock were in order. The second reason for performing Trial 1 stemmed from a hypothesis at the time that settling basins with tube-settler media might be the optimal solids removal device for pairing with the CCMB. It was thought that the desired three organic loading rates could all be achieved with the tube-settlers by varying the fish feed rate. However, it became apparent after increasing the feed rate from 400 g/day to 1,000 g/day following the completion of Trial 1 that the tube settlers were too easily clogged with solids, too easily biofouled, and difficult to clean. In addition, by the end of Trial 2 colonies of invasive *bryozoa* had infested the tube-settler media. The *bryozoa* made cleaning the tube settlers impossible without physically removing the media and washing with a high-pressure hose.

By the end of Trial 3 a lack of time and resources forced a shift from sampling at steady-state conditions once a day for five days (N=5) during Trials 1, 2, and 3 to sampling every four hours over a twenty-four hour period (N=6) for Trial 4. The change in sampling protocol was justified as the average of the water quality measurements over the diurnal period agree closely with the values measured at 10:00 am, the sampling time used during Trials 1, 2, and 3.

Following the successful completion of Trial 4 and the realization of how much quicker additional trials could be completed with the diurnal sampling protocol, an *a priori* decision was made to keep the configuration used in Trial 4 (no solids removal) and perform two additional trials whereby only the flow rate through the CCMB would

be altered between trials. Trial 5 was completed in this manner just as the environmental controls for the building that housed the experimental systems failed. The resulting daily temperature swings in excess of 4.7 ºC (10 ºF) delayed an anticipated Trial 6 beyond existing time constraints.

**4.9 Preliminary Media Investigation**

Before this research the CCMB had only ever been operated with one particular type of media made of recycled polypropylene and is identified as the gray pellets or Media 1 in this study. The first choice for an alternative media type to evaluate against the original media was a tube-type media similar to that evaluated by Sastry (1996) as it was hypothesized that the tubes would provide more effective surface area for nitrification than the original CCMB media and that the turbulence of the CCMB would keep the internal tube spaces from biofouling. The tubes were ordered and placed in a CCMB only to discover during the initial shakedown period that the tubes would not circulate and another media type had to be substituted. From this lesson it was learned that two physical characteristics of particular importance to the CCMB media are buoyancy and bulk porosity. A media with too great a porosity will not offer enough resistance to be "pushed" by the water through the recirculation pattern within the CCMB. Buoyancy is important in that if too low, the media will not rise out of the water to contact air and if too high, the media will rise so high as to stop the recirculation pattern within the CCMB. Buoyancy and porosity can be related to one another since a media with a large porosity will have less material and thus a lower buoyancy than a media with a lower porosity and the same (material) specific gravity.

# CHAPTER 5

# CONCLUSIONS

The effects of media selection and organic loading on nitrification rates in a reversed-flow, three-phase, spouted-bed, bioreactor with draft-tube (CCMB) were studied. Experiments were conducted on three identical recirculating aquaculture systems (RAS) each having a CCMB unit with a unique plastic pelletized media and operated over five successive trials, including three trials (Trials 2, 3, & 4) where components of the solids removal devices were removed after each trial in order to produce three distinct levels of organic loading. Upon analysis of the results, three levels of organic loading were not achieved and Trials 2 and 3 were combined to provide a lower organic loading (average 91 mg/L as COD) data set to compare against the higher organic loading (average 149 mg/L as COD) data from Trial 4.

## 5.1 Media Type

Comparing nitritation and nitratation rates against media type by organic loading trial groupings showed that media selection made a significant difference on nitrification performance. At both levels of organic loading, the nitritation and nitratation rates of Media 1 outperformed the other two media types. The differences between the Media 1 rates and those of Media 2 and Media 3 were more significant at the lower organic loading than at the higher loading, for nitratation than for nitritation, and for areal comparisons than for volumetric comparisons.

The differences between Media 2 and Media 3 were not statistically significant at any level of comparison. Media 2 nitritation rates were slightly higher than Media 3 rates with the difference being more pronounced at the lower organic loading than the higher and for areal comparisons than volumetric comparisons. In comparing

nitratation rates, Media 3 rates were slightly higher than Media 2 rates with a more pronounced difference at the higher organic loading than at the lower loading and for volumetric comparisons than areal comparisons.

While no explanation can be offered for the better performance of Media 1, potential explanations can be offered for the poorer performances of Media 2 and Media 3. Media 2 possessed the highest sphericity of the three media types, which may have created smaller points of impact with greater biofilm impact stresses resulting in higher particle to particle abrasion losses in the highly turbulent CCMB. Also, unlike Media 1 and Media 3, Media 2 pellets were observed to continuously shift and grind against one another while cycling through the CCMB annulus and likely producing greater abrasive biofilm losses. Conversely, the poorer performance of Media 3 might be explained by its apparent persistent hydrophobic nature. This characteristic was also suspected as the cause of an apparent delayed acclimation period in System3, where the extreme hydrophobic nature of the media may have retarded the rate of biofilm attachment and increased the rate of biofilm detachment.

Another difference between media types was observed with the out-of-scope increase in flow rate between Trial 4 and Trial 5. A significant increase in system COD occurred when the flow rates were increased that could only be explained by a reduction in the CCMB static-zone settling efficiency due to increased internal recirculation rates and media velocity. Because the different media required different circulation rates to flow properly, media type may explain the significantly different COD increases between systems as a media geometry capable of slower cycling might allow more solids settling.

During pre-trial shakedown, a particular media type chosen for this study failed to hydraulically operate in the CCMB. This revealed that two physical characteristics of particular importance to the CCMB media are buoyancy and bulk porosity. A media with too great a porosity or with too low a buoyancy will not flow through the recirculation pattern of the CCMB.

**5.2 Organic Loading**

Comparing nitritation and nitratation rates against the organic loading (measured as COD) trial groupings for each media type showed that organic loading made a much less significant difference in nitrification performance than did media type in the earlier comparison. A barely significant difference between nitrification rates at the two organic loadings was observed only for Media 1 nitratation, where the nitratation rate was greater at the lower organic loading. There was no difference in the statistical comparisons between volumetric and areal nitrification rates at two organic loadings for the same media because the only difference between the volumetric and areal data sets was the surface area to volume ratio, a constant for each media type.

The minimal decrease in nitrification rates observed for Media 1 as organic loading increased, could be attributed to the discussion presented in Section 2.5.7, that heterotrophs are capable of greater competition with nitrifiers as organic loading increases, but with the effect being less pronounced for thin biofilm systems.

**5.3 Nitrification Rate Summary**

Throughout all trials, the CCMB demonstrated the ability to successfully nitrify over organic levels ranging from 13.5 to 205.3 mg/L COD (see Table 4.1.4-1) and without showing any signs of biofouling or other problems associated with traditional fixed-film nitrification systems. The original CCMB media (Media 1) achieved the

highest nitritation rates during all trials (see Table 5.3-1 and Section C.1) with an average $C_A^{V'}$ of 223 g TAN/day-m$^3$ (0.0139 lb TAN/day-ft$^3$) and maximum of 254 g TAN/day-m$^3$ (0.0159 lb TAN/day-ft$^3$) observed during Trial 5, which had both the highest organic loading and flow rates.

Table 5.3-1. Mean nitrification rates and COD loading of all trials by media system.

| Media | Trial | Mean $C_A^{V'}$ | Mean $C_N^{V'}$ | Mean $C_A^{A'}$ | Mean $C_N^{A'}$ | Mean COD |
|---|---|---|---|---|---|---|
| | | g TAN/day-m$^3$ (lb TAN/day-ft$^3$) | | mg TAN/day-m$^2$ (lb TAN/day-ft$^2$ x10$^{-5}$) | | mg/L |
| 1 | 1 | 167 (0.0105) | 182 (0.0114) | 153 (3.14) | 166 (3.40) | 21.9 |
| | 2 | 228 (0.0142) | 347 (0.0216) | 208 (4.26) | 317 (6.49) | 94.4 |
| | 3 | 247 (0.0154) | 279 (0.0174) | 225 (4.62) | 255 (5.23) | 105 |
| | 4 | 217 (0.0135) | 241 (0.0150) | 198 (4.06) | 220 (4.51) | 147 |
| | 5 | 254 (0.0159) | 308 (0.0192) | 232 (4.76) | 282 (5.77) | 205 |
| 2 | 1 | 155 (0.0097) | 160 (0.0100) | 134 (2.75) | 138 (2.83) | 21.9 |
| | 2 | 176 (0.0110) | 171 (0.0106) | 152 (3.11) | 148 (3.02) | 83.7 |
| | 3 | 174 (0.0109) | 174 (0.0108) | 151 (3.09) | 150 (3.08) | 82.9 |
| | 4 | 162 (0.0101) | 173 (0.0108) | 140 (2.87) | 150 (3.07) | 153 |
| | 5 | 187 (0.0117) | 199 (0.0124) | 162 (3.31) | 172 (3.53) | 198 |
| 3 | 1 | 156 (0.0098) | 161 (0.0100) | 128 (2.63) | 132 (2.70) | 13.5 |
| | 2 | 148 (0.0092) | 169 (0.0106) | 121 (2.48) | 139 (2.84) | 90.2 |
| | 3 | 187 (0.0117) | 207 (0.0129) | 154 (3.14) | 169 (3.47) | 87.2 |
| | 4 | 160 (0.0100) | 195 (0.0121) | 131 (2.69) | 160 (3.27) | 146 |
| | 5 | 185 (0.0115) | 228 (0.0142) | 152 (3.11) | 187 (3.83) | 173 |

# CHAPTER 6

# RECOMMENDATIONS

The effect of organic loading on nitrification has been demonstrated to have little effect on the nitrification rates of the CCMB from 91 mg/L COD to 149 mg/L COD and no further research on this topic is necessary. However, based on the observed effects of media type and flow rate on nitrification performance, future research should be conducted on these parameters in order to optimize CCMB performance.

It is highly doubtful that the first media used in the CCMB (Media 1) and selected solely on availability would be the best performing media possible. Future experiments to select the optimum media might

1. use similarly shaped media but with different plastics and or additives (i.e., hydrophobic) to determine the effect of media composition; or
2. use media with different shapes or surface roughness but with the same composition to determine the effect of shear and abrasion losses, or conversely biofilm thickness.

The 17% increase in mean nitritation, 28% increase in mean nitratation, and inferred 40% decrease in COD removal observed after the out-of-scope increase in flow rate between Trials 4 and 5 for Media 1 provide a strong argument for research into discovering the optimal CCMB flow rate. Future research should

1. determine the impact of flow on CCMB nitrification rates;
2. weigh the economics of power consumption against any gains in nitrification realized by increasing flow; and
3. determine the relationship between CCMB internal recirculation rates and solids settling for COD removal.

Considering the debate into the identification of nitrifying microorganisms discussed in Section 2.3, future research should include the exact identification of the microorganisms responsible for nitrification in the CCMB so that the performance of the CCMB can be better evaluated among different systems. The following questions might be considered in future research.

1. Which microorganisms are responsible for nitrification in the CCMB?
2. How do these microorganisms compare to the predominant nitrifying microorganisms in other fixed-film systems?
3. What physical or kinetic variables might cause population shifts within the CCMB biofilm?
4. Is it feasible to seed a CCMB system with known microorganisms of known kinetic rates and expect those microorganisms to both remain and dominate the CCMB biofilm?

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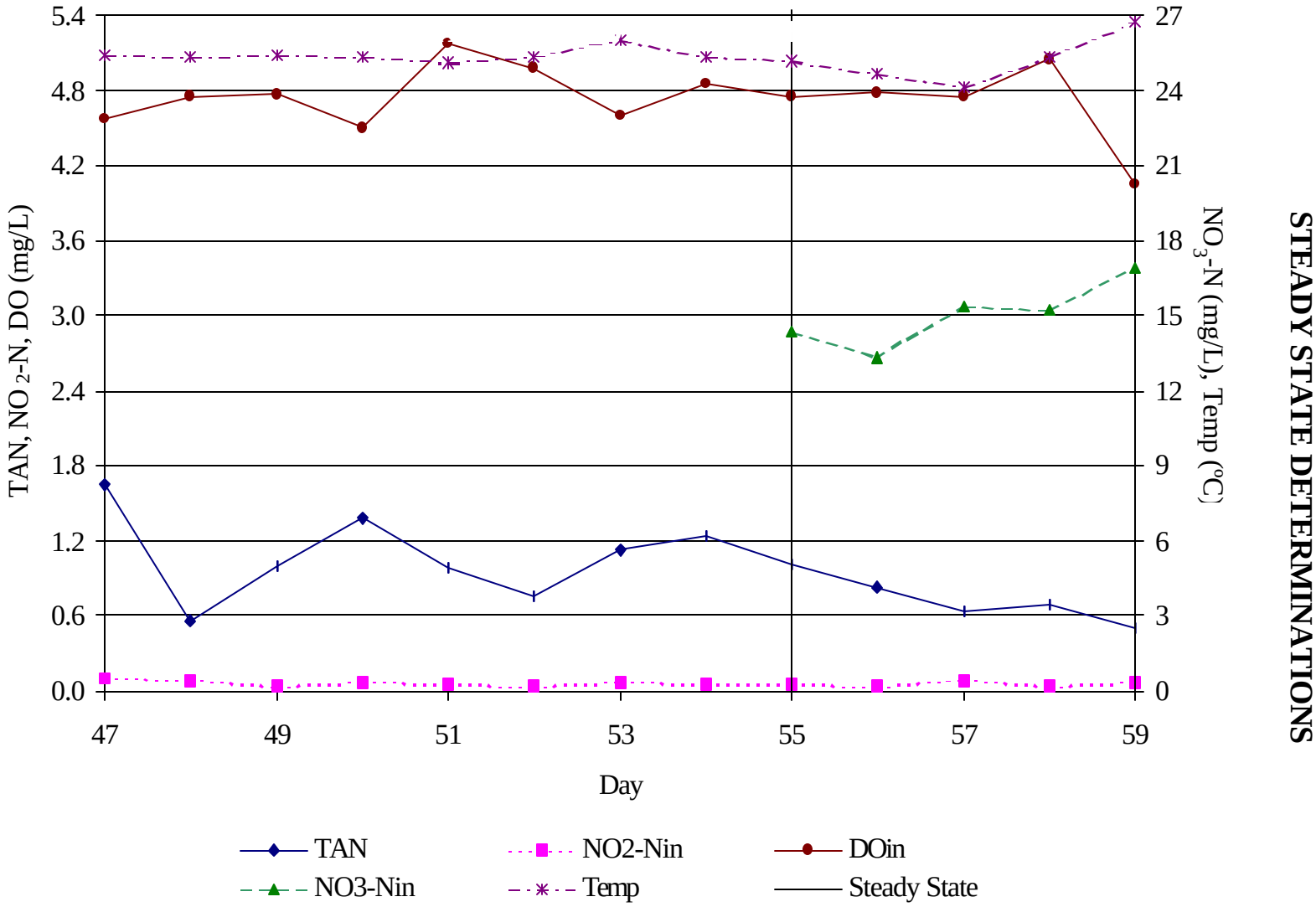



Figure A-1. Steady-state determination System 1 Trial 1.

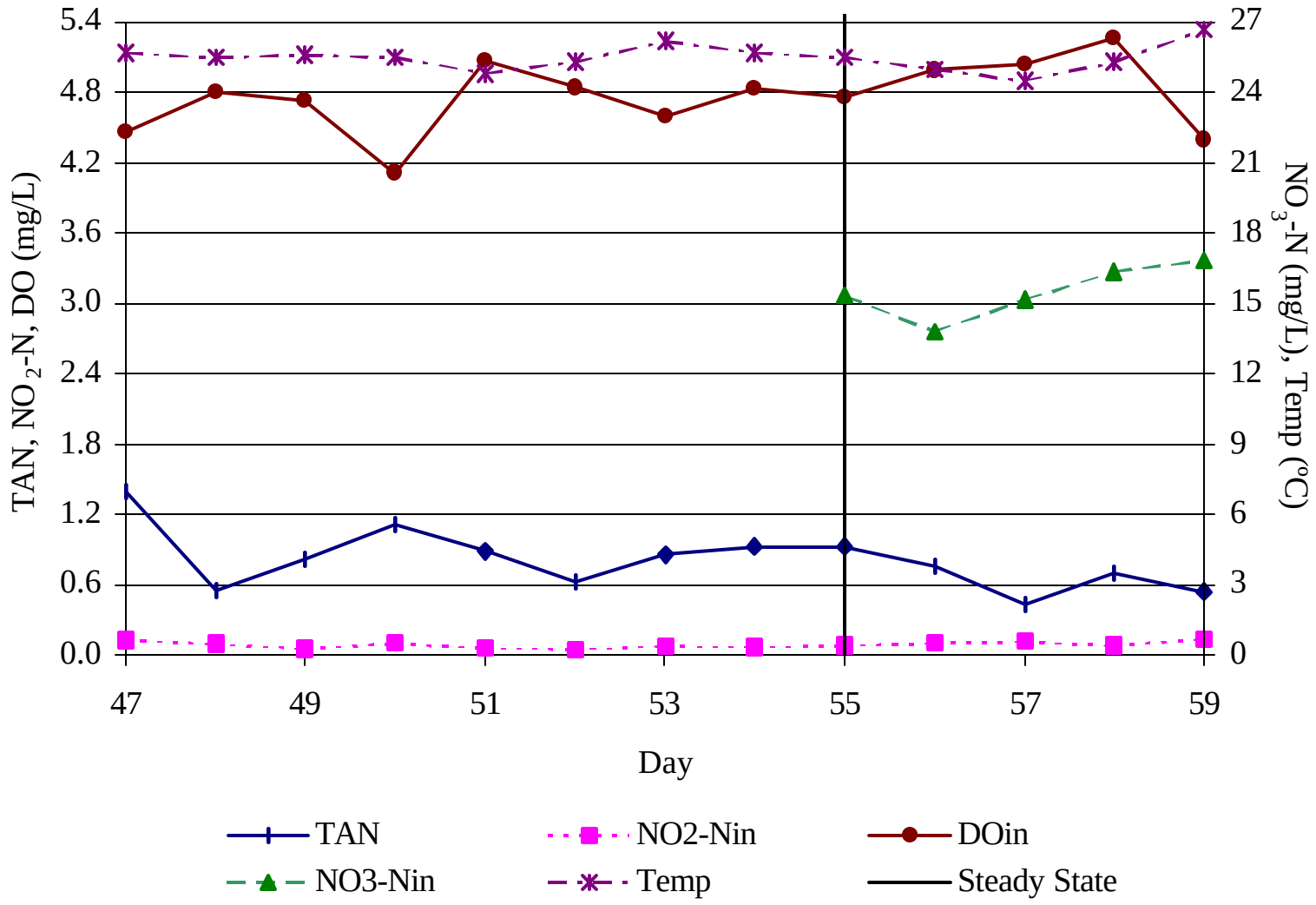



Figure A-2. Steady-state determination System 2 Trial 1.

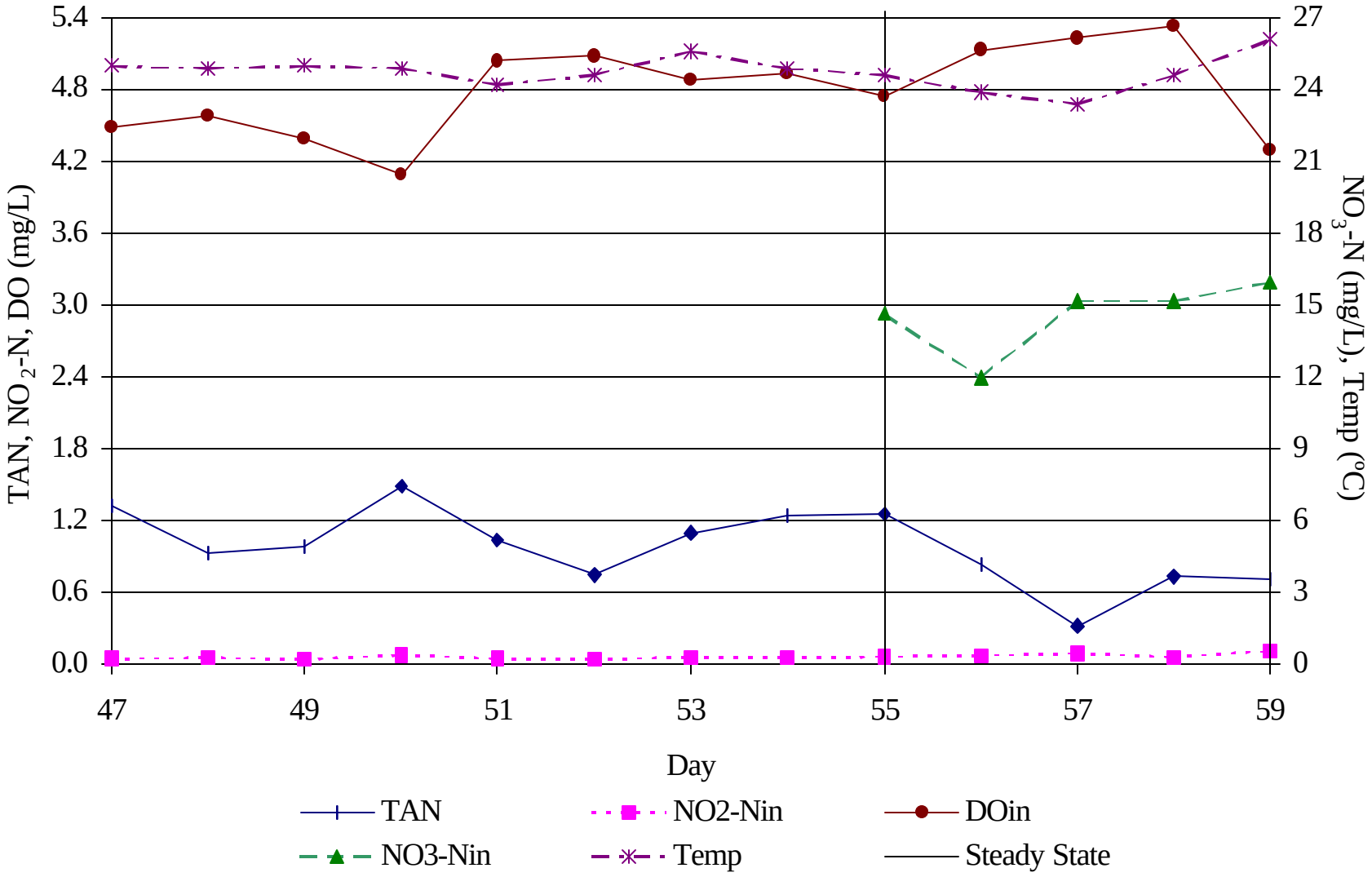



Figure A-3. Steady-state determination System 3 Trial 1.

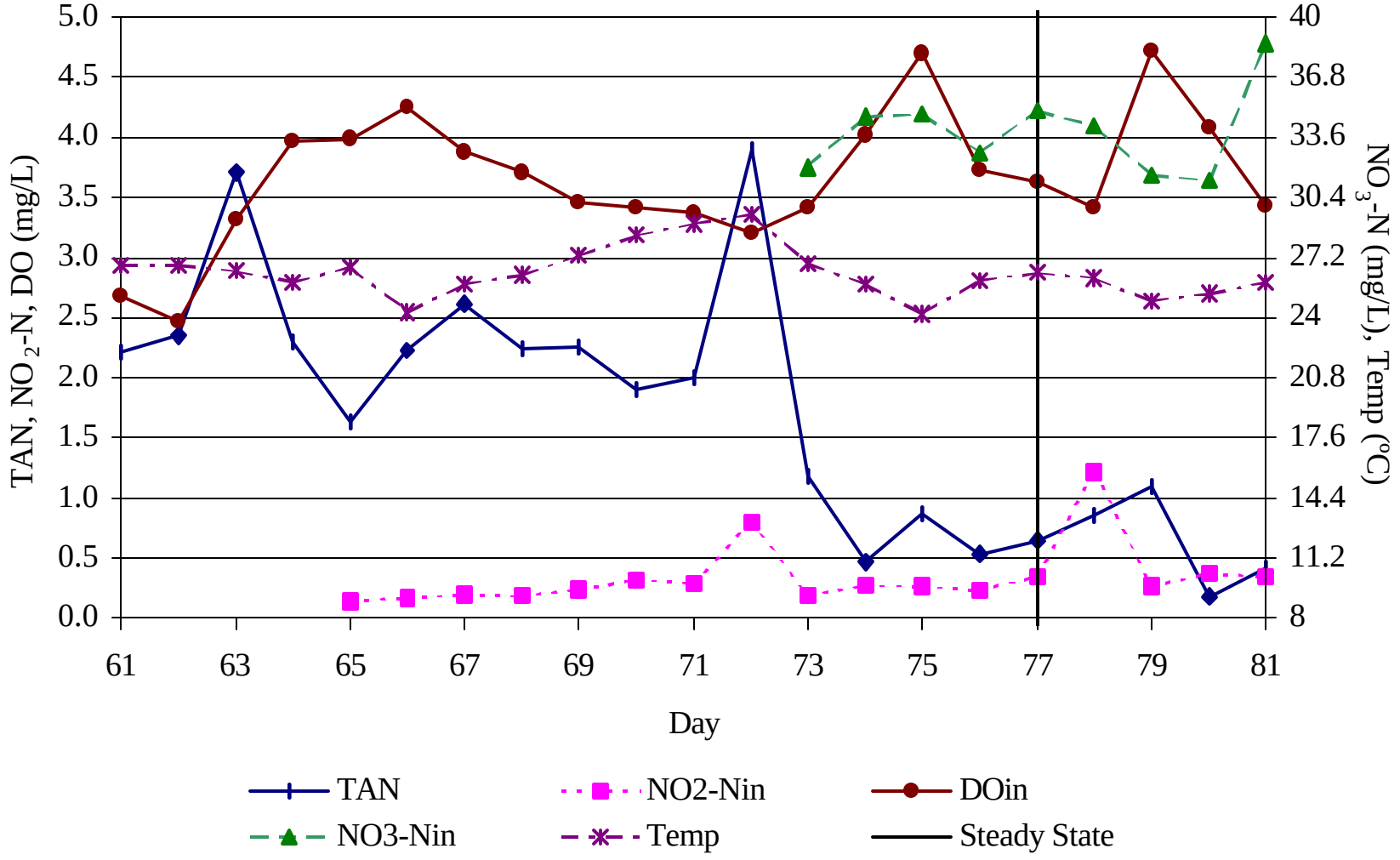
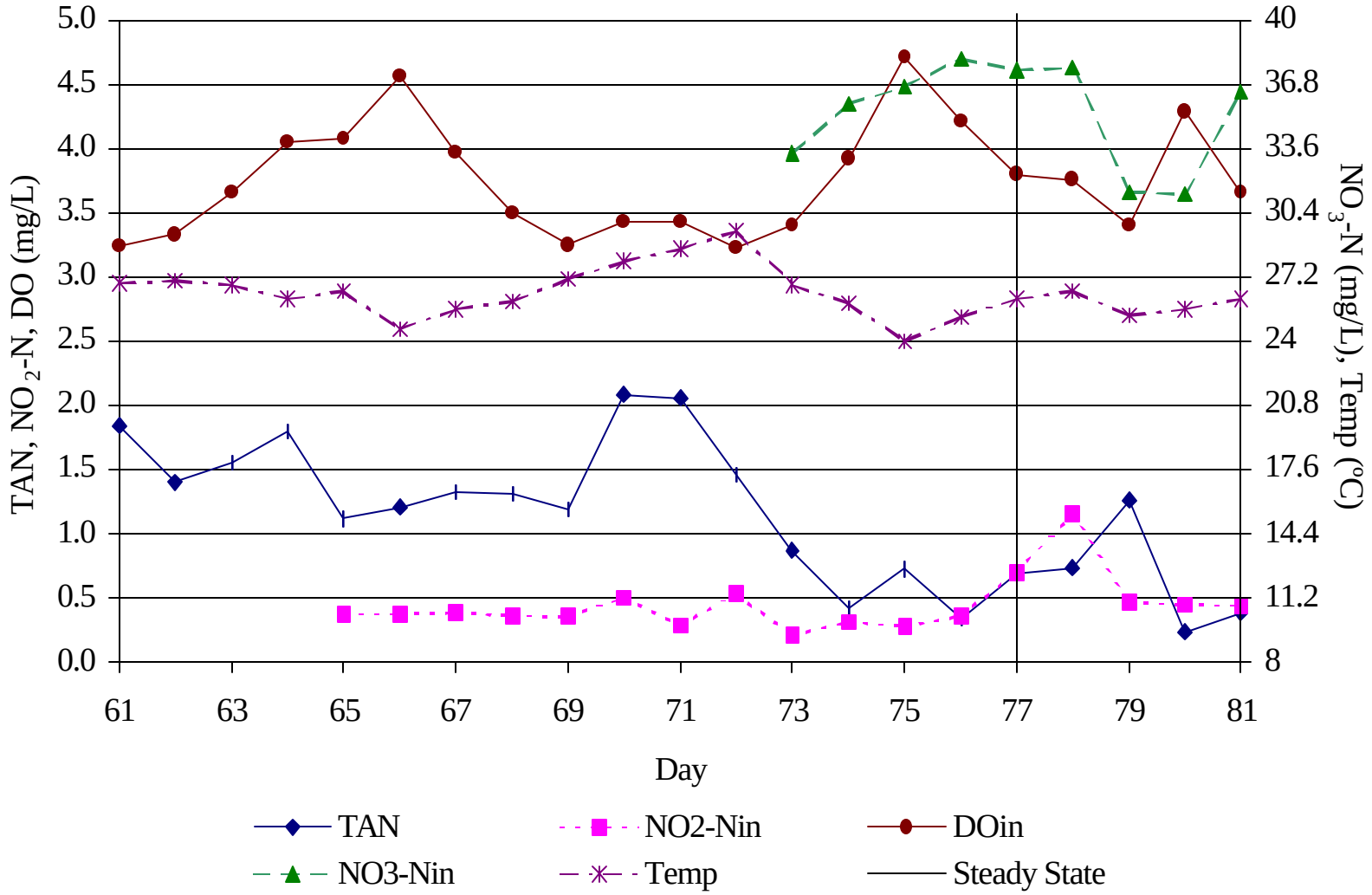



Figure A-4. Steady-state determination System 1 Trial 2.

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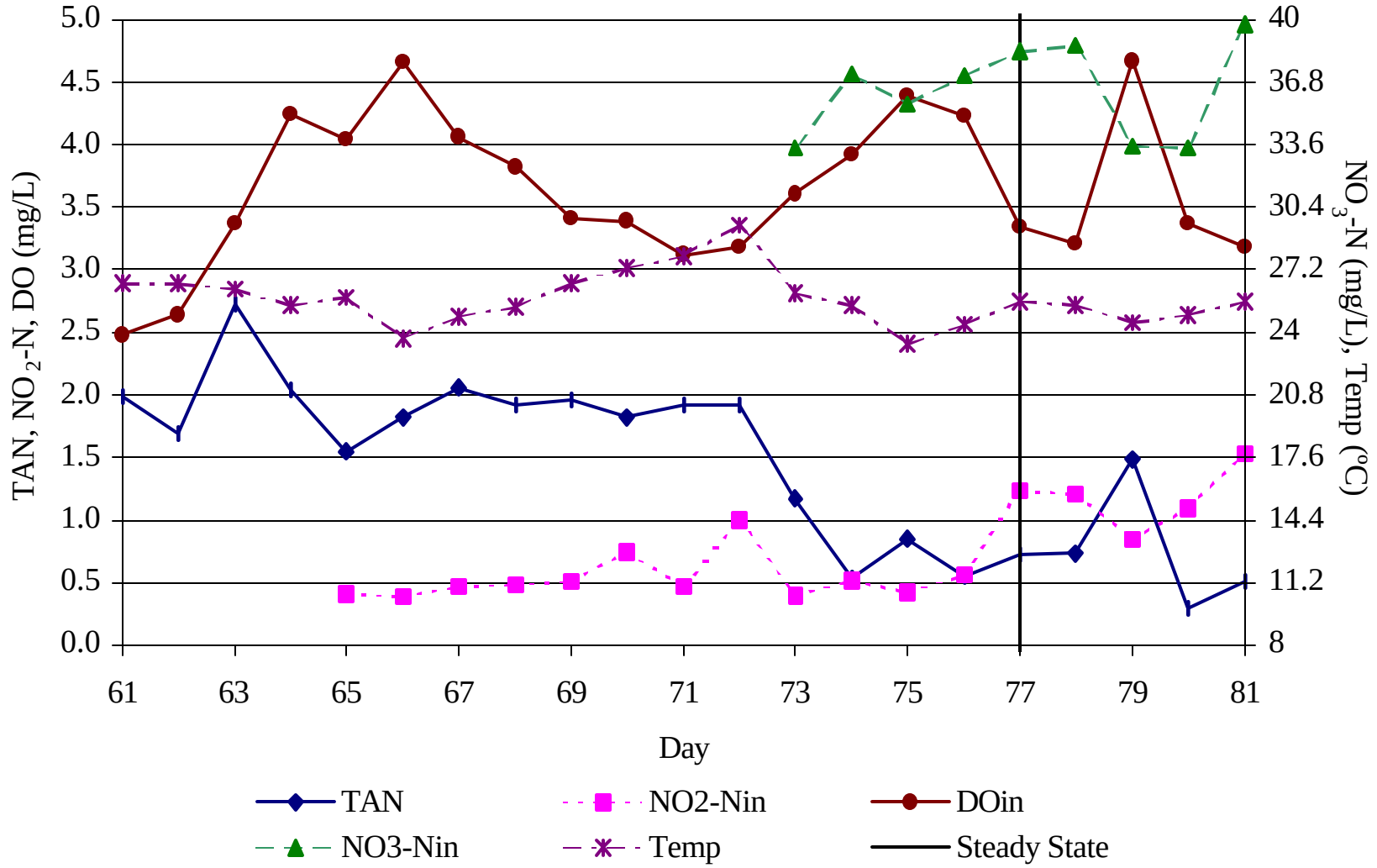


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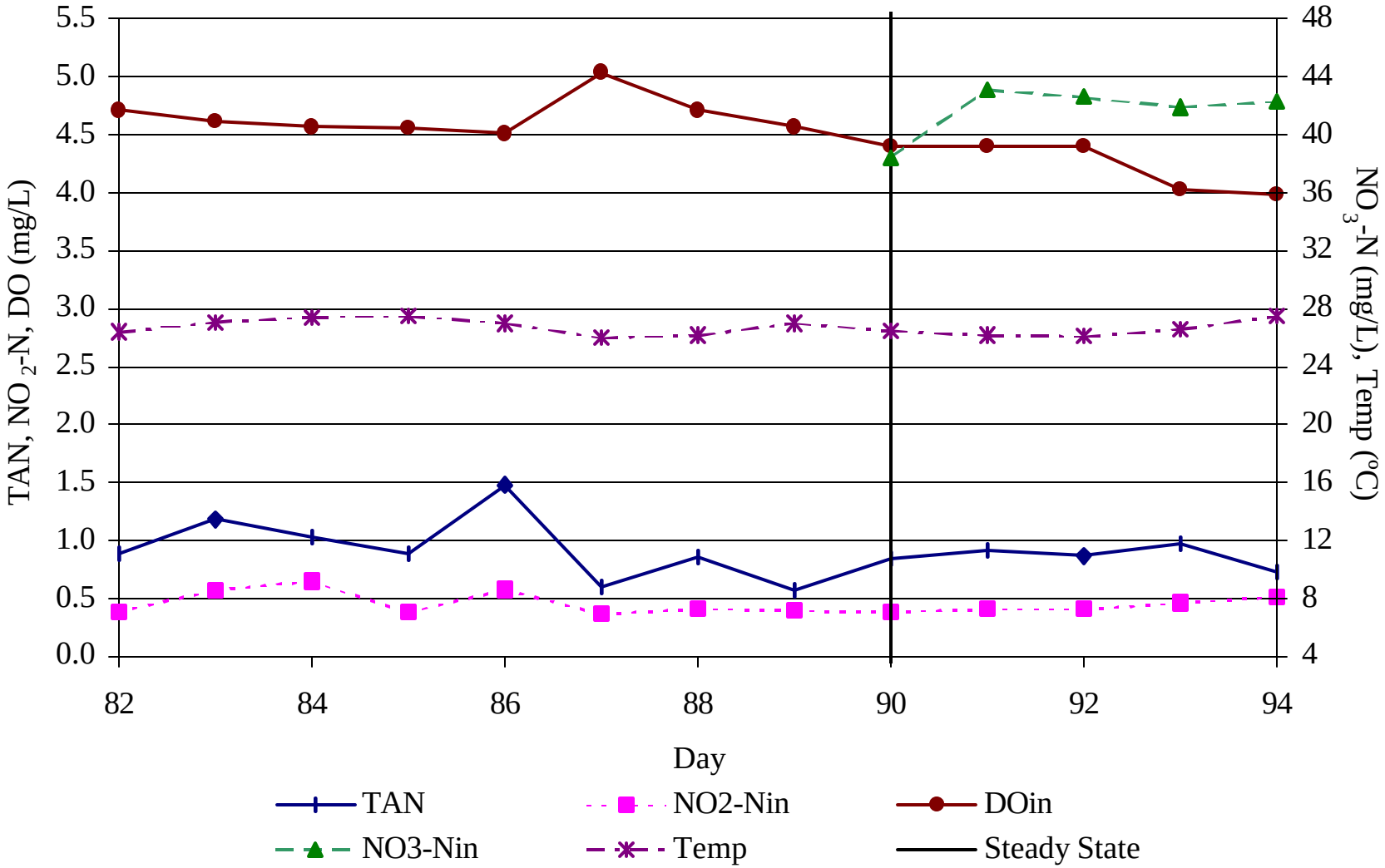
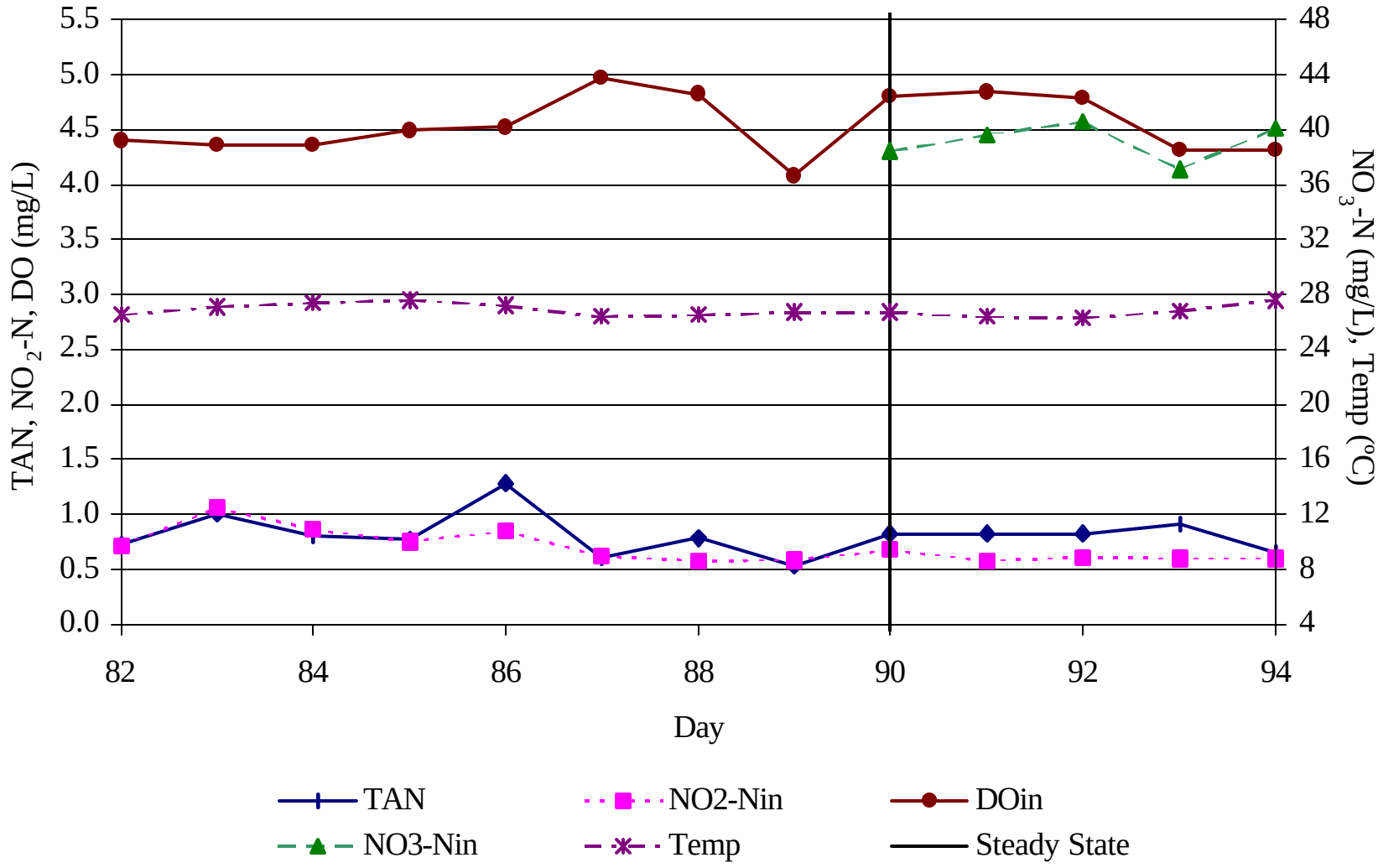



Figure A-7. Steady-state determination System 1 Trial 3.

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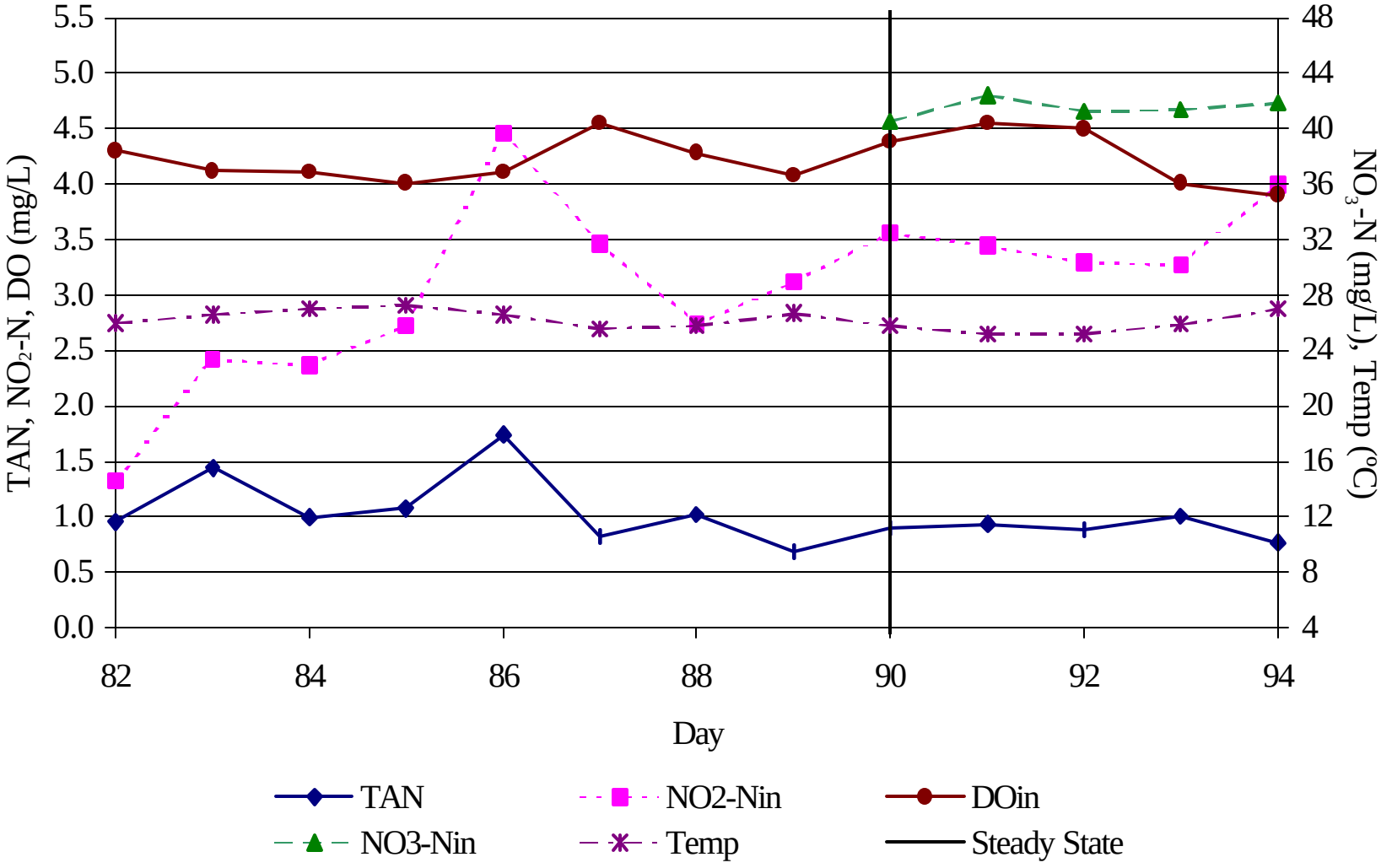


Figure A-9. Steady-state determination System 3 Trial 3.

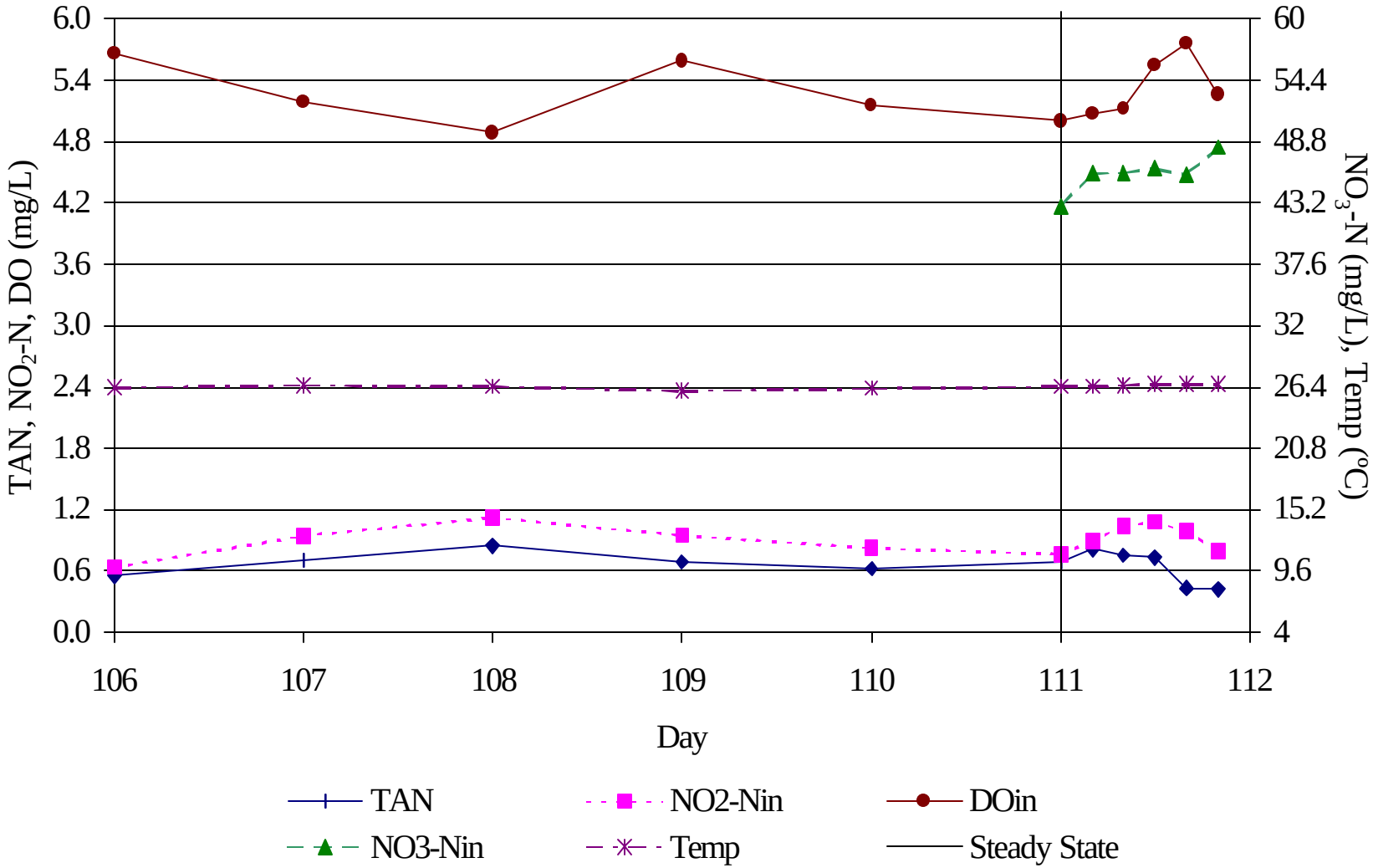


Figure A-10. Steady-state determination System 1 Trial 4.

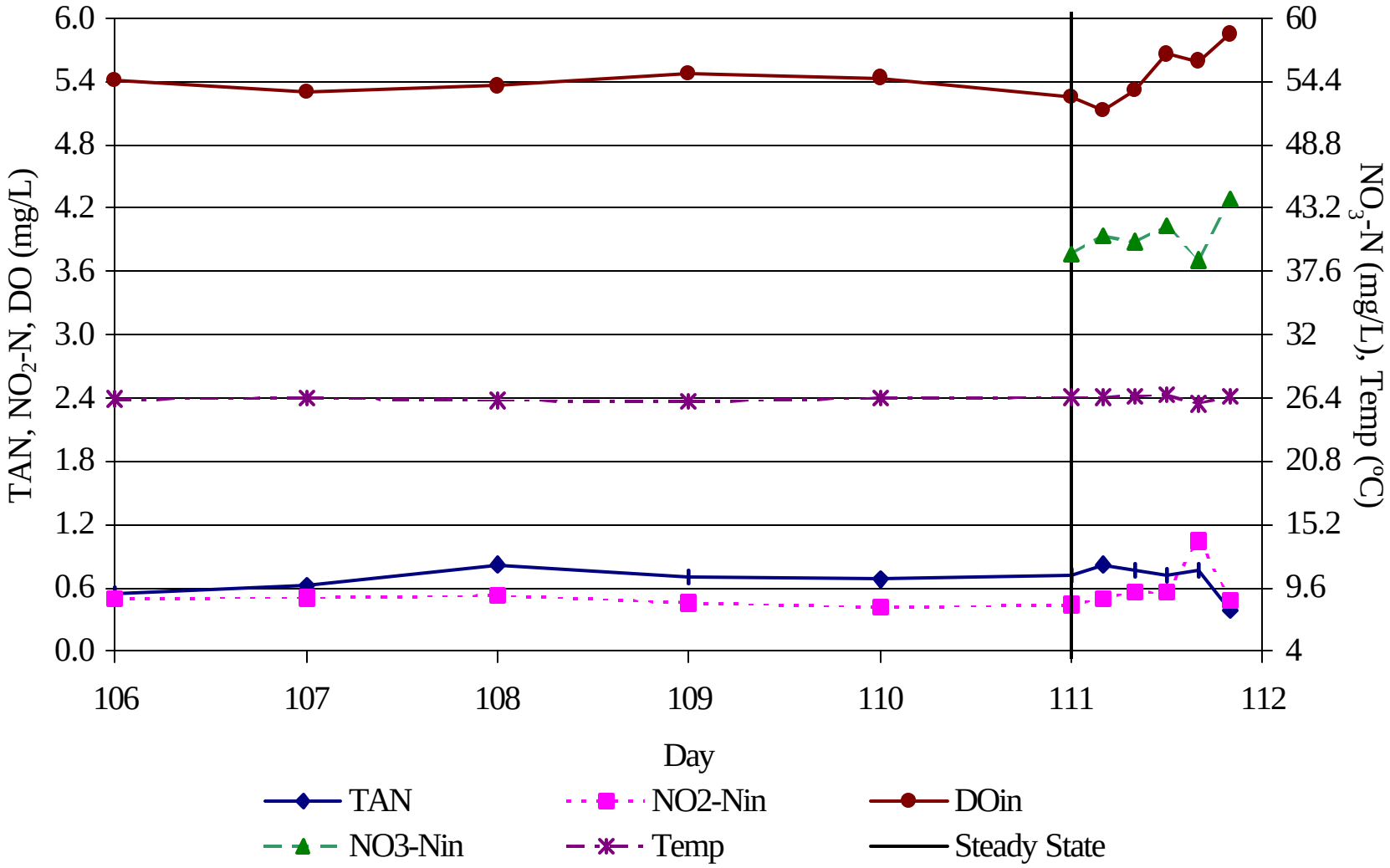


Figure A-11. Steady-state determination System 2 Trial 4.

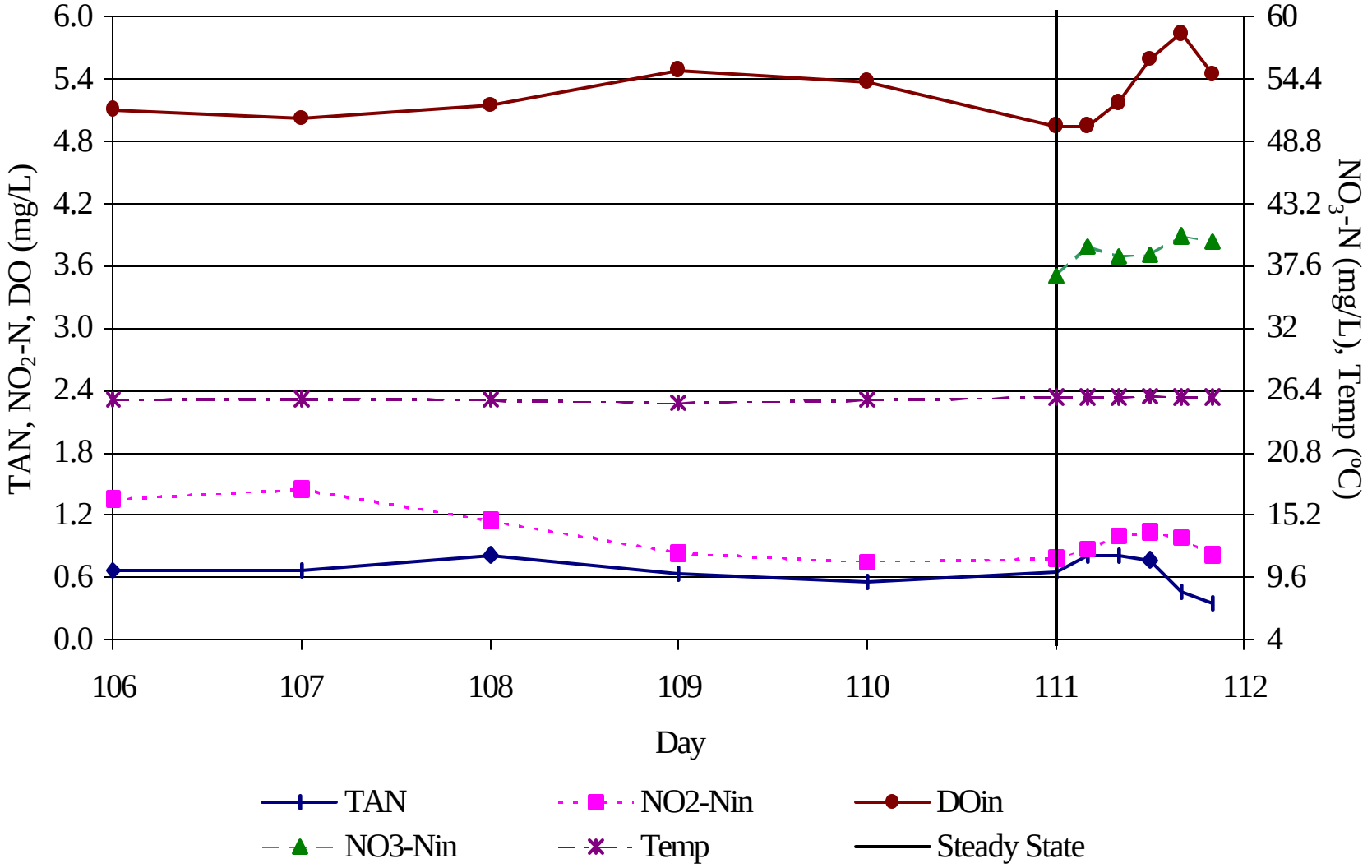


Figure A-12. Steady-state determination System 3 Trial 4.

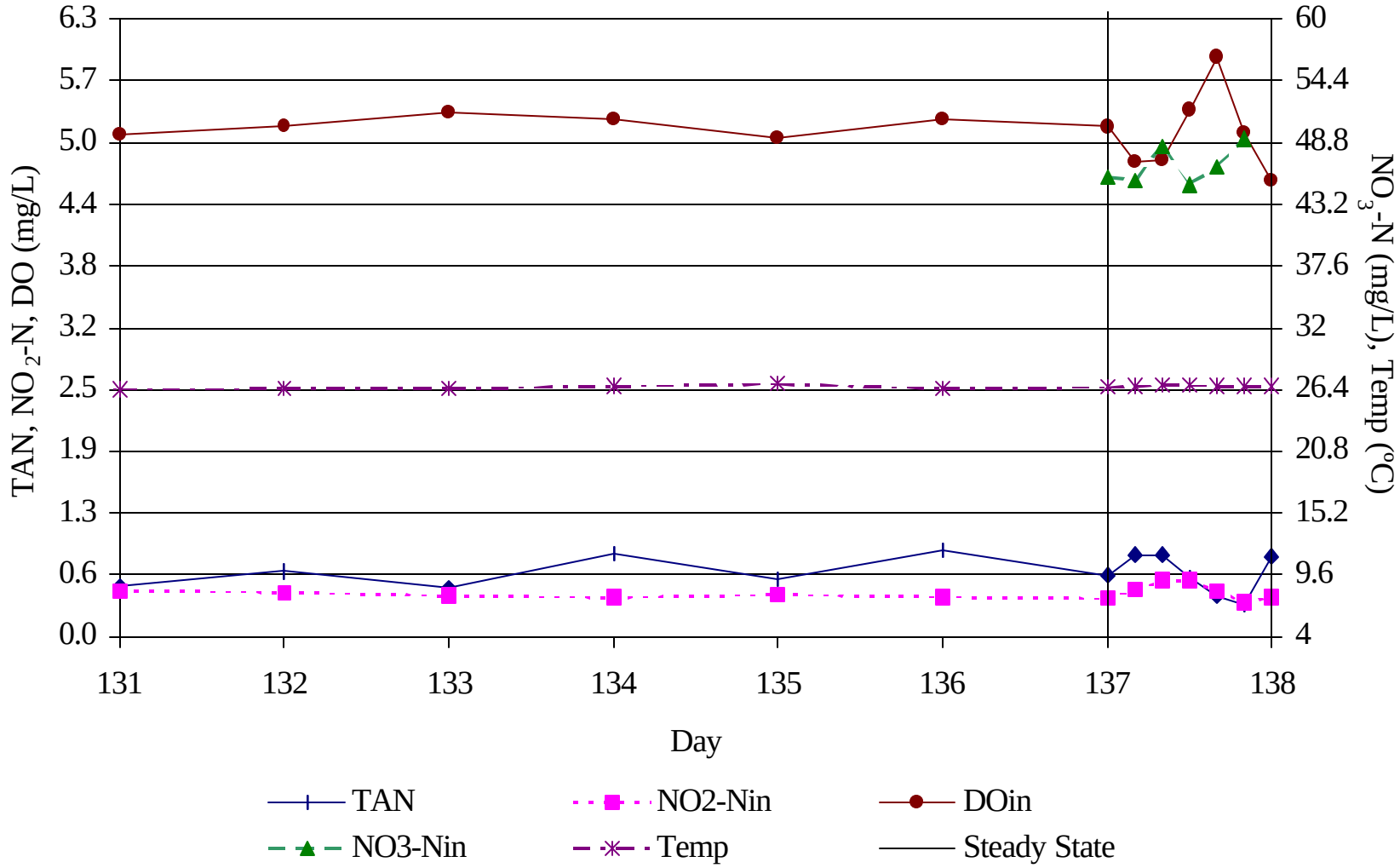


Figure A-13. Steady-state determination System 1 Trial 5.

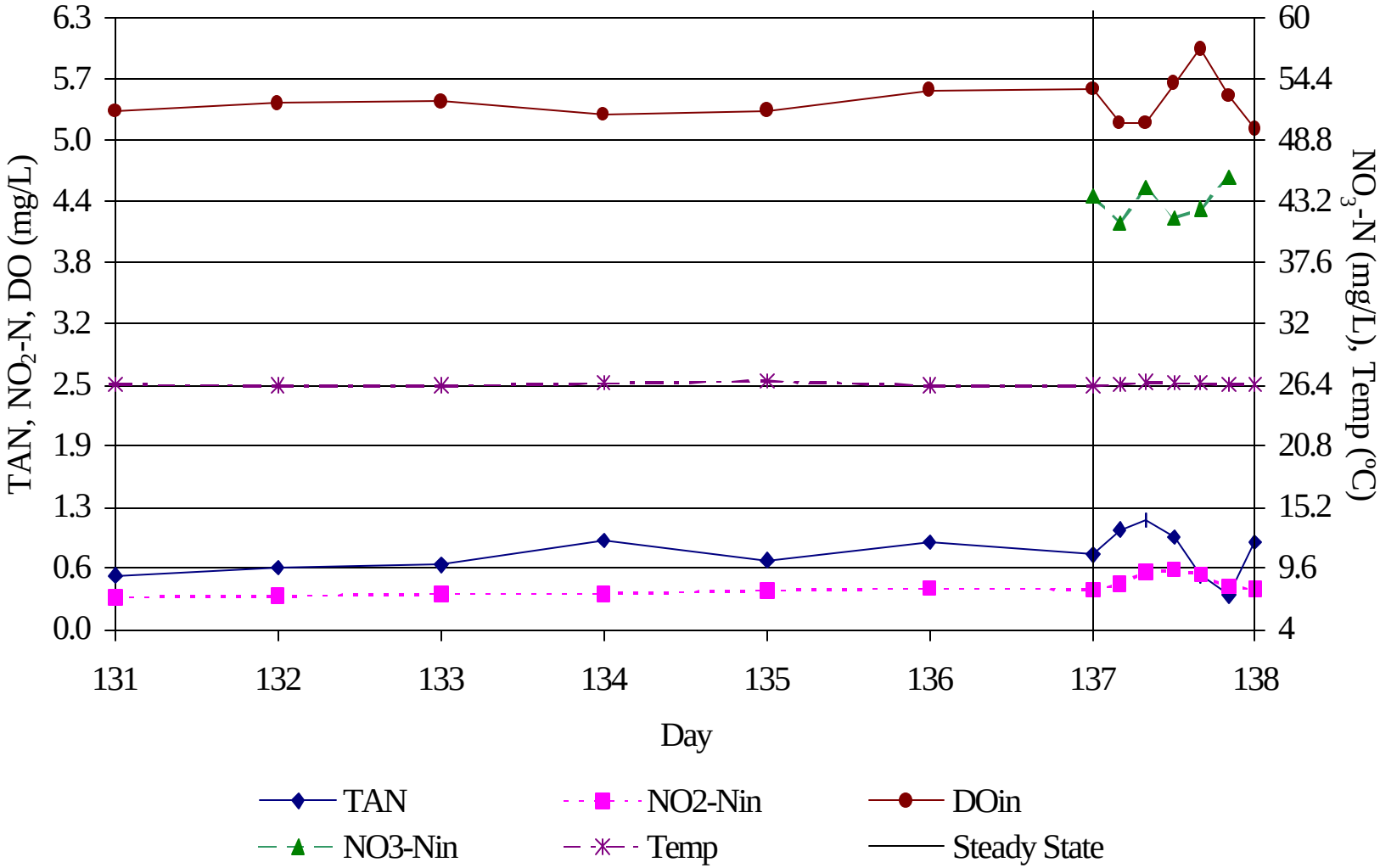


Figure A-14. Steady-state determination System 2 Trial 5.

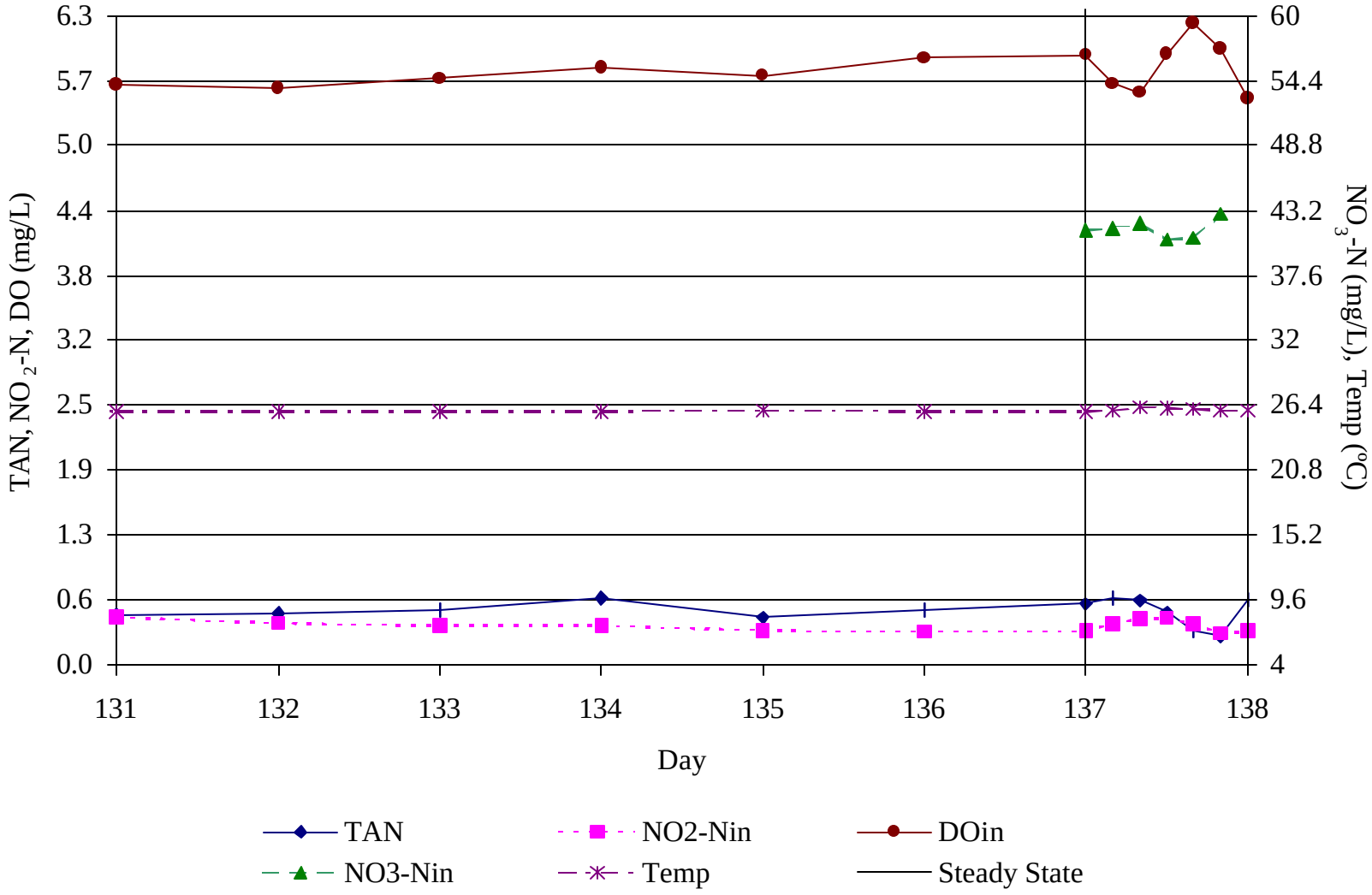


Figure A-15. Steady-state determination System 3 Trial 5.

## APPENDIX B.

## PAIRED DATA EVALUATIONS

Table B-1. Paired data evaluations and comparisons of means.

| Parameter | Media | Trial | N | Mean | St Dev | T | p |
|---|---|---|---|---|---|---|---|
| TAN | 1 | 1 | 5 | 0.1946 | 0.0959 | 4.54 | 0.0105 |
| | | 2 | 5 | 0.2206 | 0.1197 | 4.12 | 0.0146 |
| | | 3 | 5 | 0.3194 | 0.0263 | 27.16 | <0.0001 |
| | | 4 | 6 | 0.2008 | 0.0361 | 13.64 | <0.0001 |
| | | 5 | 6 | 0.1642 | 0.07 | 5.74 | 0.0022 |
| | | 2+3 | 10 | 0.27 | 0.0969 | 8.81 | <0.0001 |
| | 2 | 1 | 5 | 0.1626 | 0.0741 | 4.91 | 0.008 |
| | | 2 | 5 | 0.179 | 0.1243 | 3.22 | 0.0323 |
| | | 3 | 5 | 0.2108 | 0.021 | 22.48 | <0.0001 |
| | | 4 | 6 | 0.167 | 0.0362 | 11.29 | <0.0001 |
| | | 5 | 6 | 0.148 | 0.0384 | 9.44 | 0.0002 |
| | | 2+3 | 10 | 0.1949 | 0.0857 | 7.19 | <0.0001 |
| | 3 | 1 | 5 | 0.1972 | 0.1289 | 3.42 | 0.0268 |
| | | 2 | 5 | 0.1654 | 0.0955 | 3.87 | 0.0179 |
| | | 3 | 5 | 0.2512 | 0.0243 | 23.13 | <0.0001 |
| | | 4 | 6 | 0.145 | 0.0279 | 12.73 | <0.0001 |
| | | 5 | 6 | 0.0943 | 0.0255 | 9.08 | <0.0001 |
| | | 2+3 | 10 | 0.2083 | 0.0797 | 8.26 | <0.0001 |
| NO2-N | 1 | 1 | 5 | 0.0024 | 0.0084 | 0.64 | 0.5583 |
| | | 2 | 5 | 0.0656 | 0.056 | 2.62 | 0.0587 |
| | | 3 | 5 | 0.0214 | 0.0107 | 4.48 | 0.011 |
| | | 4 | 6 | 0.033 | 0.0123 | 6.58 | 0.0012 |
| | | 5 | 6 | 0.0245 | 0.0164 | 3.67 | 0.0145 |
| | | 2+3 | 10 | 0.0435 | 0.0446 | 3.09 | 0.013 |
| | 2 | 1 | 5 | -3.00E-18 | 0.0103 | 0 | 1 |
| | | 2 | 5 | 0.0024 | 0.0273 | 0.2 | 0.8539 |
| | | 3 | 5 | -2.00E-04 | 0.0145 | -0.03 | 0.9769 |
| | | 4 | 6 | 0.0128 | 0.0201 | 1.56 | 0.1787 |
| | | 5 | 6 | 0.0068 | 0.0067 | 2.49 | 0.0555 |
| | | 2+3 | 10 | 0.0011 | 0.0207 | 0.17 | 0.8701 |
| | 3 | 1 | 5 | 0.0014 | 0.0052 | 0.6 | 0.5814 |
| | | 2 | 5 | 0.0394 | 0.0347 | 2.54 | 0.064 |
| | | 3 | 5 | 0.0998 | 0.0247 | 9.03 | 0.0008 |
| | | 4 | 6 | 0.0468 | 0.0141 | 8.15 | 0.0005 |
| | | 5 | 6 | 0.0173 | 0.0142 | 2.79 | 0.0303 |
| | | 2+3 | 10 | 0.0696 | 0.0427 | 5.16 | 0.0006 |

Table B-1. Continued.

| Parameter | Media | Trial | N | Mean | St Dev | T | p |
|---|---|---|---|---|---|---|---|
| NO3-N | 1 | 1 | 5 | 0.34 | 1.2315 | 0.62 | 0.5704 |
| | | 2 | 5 | -0.793 | 1.1091 | -1.6 | 0.185 |
| | | 3 | 5 | 0.7798 | 1.4251 | 1.22 | 0.2883 |
| | | 4 | 6 | 1.3502 | 1.9926 | 1.66 | 0.1579 |
| | | 5 | 6 | 0.189 | 1.2424 | 0.37 | 0.7247 |
| | | 2+3 | 10 | -0.007 | 1.4618 | -0.01 | 0.9886 |
| | 2 | 1 | 5 | 0.2622 | 0.7819 | 0.75 | 0.495 |
| | | 2 | 5 | -0.054 | 1.2071 | -0.1 | 0.9251 |
| | | 3 | 5 | 0.8334 | 1.6036 | 1.16 | 0.3098 |
| | | 4 | 6 | 0.5943 | 1.8528 | 0.79 | 0.4676 |
| | | 5 | 6 | -0.35 | 1.1632 | -0.74 | 0.494 |
| | | 2+3 | 10 | 0.3897 | 1.4174 | 0.87 | 0.4072 |
| | 3 | 1 | 5 | -0.06 | 0.5261 | -0.25 | 0.8125 |
| | | 2 | 5 | -1.573 | 1.288 | -2.73 | 0.0524 |
| | | 3 | 5 | -0.08 | 0.9988 | -0.18 | 0.8666 |
| | | 4 | 6 | 0.3058 | 0.8642 | 0.87 | 0.4257 |
| | | 5 | 6 | 0.0113 | 0.5519 | 0.05 | 0.9618 |
| | | 2+3 | 10 | -0.827 | 1.3417 | -1.95 | 0.0832 |
| COD | 1 | 1 | 5 | 1.8 | 3.5637 | 1.13 | 0.3219 |
| | | 2 | 5 | 4.8 | 3.7014 | 2.9 | 0.0441 |
| | | 3 | 5 | -1.4 | 5.5045 | -0.57 | 0.6 |
| | | 4 | 6 | -5.167 | 1.7224 | -7.35 | 0.0007 |
| | | 5 | 6 | 1.8333 | 13.303 | 0.34 | 0.7494 |
| | | 2+3 | 10 | 1.7 | 5.4985 | 0.98 | 0.3538 |
| | 2 | 1 | 5 | 4.6 | 5.4589 | 1.88 | 0.1326 |
| | | 2 | 5 | -0.2 | 11.122 | -0.04 | 0.9699 |
| | | 3 | 5 | -2.2 | 4.2071 | -1.17 | 0.3072 |
| | | 4 | 6 | -0.333 | 7.2296 | -0.11 | 0.9145 |
| | | 5 | 6 | 5.6667 | 11.843 | 1.17 | 0.294 |
| | | 2+3 | 10 | -1.2 | 7.9972 | -0.47 | 0.6464 |
| | 3 | 1 | 5 | 3.4 | 9.9146 | 0.77 | 0.4859 |
| | | 2 | 5 | -2.4 | 10.991 | -0.49 | 0.6509 |
| | | 3 | 5 | 0 | 4.3012 | 0 | 1 |
| | | 4 | 6 | 8.1667 | 6.6758 | 3 | 0.0302 |
| | | 5 | 6 | 1.8333 | 6.1128 | 0.73 | 0.4955 |
| | | 2+3 | 10 | -1.2 | 7.9694 | -0.48 | 0.6453 |
| DO | 1 | 1 | 5 | -0.436 | 0.2466 | -3.95 | 0.0168 |
| | | 2 | 5 | 0.208 | 0.1295 | 3.59 | 0.0229 |
| | | 3 | 5 | 0.556 | 0.1021 | 12.17 | 0.0003 |
| | | 4 | 6 | 0.3817 | 0.1123 | 8.32 | 0.0004 |
| | | 5 | 6 | -0.072 | 0.2525 | -0.7 | 0.5178 |
| | | 2+3 | 10 | 0.382 | 0.2138 | 5.65 | 0.0003 |

Table B-1. Continued.

| Parameter | Media | Trial | N | Mean | St Dev | T | p |
|---|---|---|---|---|---|---|---|
| DO | 2 | 1 | 5 | -0.304 | 0.0182 | -37.42 | <0.0001 |
| continued | | 2 | 5 | 0.1 | 0.0897 | 2.49 | 0.0673 |
| | | 3 | 5 | 0.438 | 0.0259 | 37.84 | <0.0001 |
| | | 4 | 6 | 0.465 | 0.0981 | 11.61 | <0.0001 |
| | | 5 | 6 | -0.003 | 0.0882 | -0.09 | 0.9299 |
| | | 2+3 | 10 | 0.269 | 0.1887 | 4.51 | 0.0015 |
| | 3 | 1 | 5 | -0.174 | 0.0709 | -5.49 | 0.0054 |
| | | 2 | 5 | 0.136 | 0.2233 | 1.36 | 0.245 |
| | | 3 | 5 | 0.642 | 0.0661 | 21.72 | <0.0001 |
| | | 4 | 6 | 0.2533 | 0.1521 | 4.08 | 0.0096 |
| | | 5 | 6 | -0.057 | 0.0789 | -1.76 | 0.1389 |
| | | 2+3 | 10 | 0.389 | 0.3086 | 3.99 | 0.0032 |
| TN | 1 | 1 | 5 | -8.34 | 17.065 | -1.09 | 0.3359 |
| | | 2 | 5 | -4.92 | 9.4785 | -1.16 | 0.3103 |
| | | 3 | 5 | -3.66 | 12.28 | -0.67 | 0.5416 |
| | | 4 | 6 | 3.8167 | 7.5774 | 1.23 | 0.2721 |
| | | 5 | 6 | -2.317 | 12.293 | -0.46 | 0.6637 |
| | | 2+3 | 10 | -4.29 | 10.363 | -1.31 | 0.2229 |
| | 2 | 1 | 5 | 1.56 | 5.2453 | 0.67 | 0.5424 |
| | | 2 | 5 | 0.34 | 12.541 | 0.06 | 0.9546 |
| | | 3 | 5 | -3.52 | 10.956 | -0.72 | 0.5122 |
| | | 4 | 6 | 6.4333 | 17.353 | 0.91 | 0.4055 |
| | | 5 | 6 | -0.333 | 11.336 | -0.07 | 0.9454 |
| | | 2+3 | 10 | -1.59 | 11.287 | -0.45 | 0.6665 |
| | 3 | 1 | 5 | 9.26 | 17.627 | 1.17 | 0.3053 |
| | | 2 | 5 | 2.34 | 3.9221 | 1.33 | 0.2531 |
| | | 3 | 5 | -7.78 | 14.114 | -1.23 | 0.2852 |
| | | 4 | 6 | 4.6667 | 13.087 | 0.87 | 0.4224 |
| | | 5 | 6 | 8.0333 | 11.987 | 1.64 | 0.1616 |
| | | 2+3 | 10 | -2.72 | 11.128 | -0.77 | 0.4594 |

## APPENDIX C.

## FILTER PERFORMANCE OF ALL TRIALS

### C.1 Nitrification Rates of Media Systems by Trial

Table C.1-1. Comparison of volumetric nitritation rates ($C_A^{V'}$) normalized for influent TAN as g TAN/day-m$^3$ (lb TAN/day-ft$^3$) of media systems by trial.

| Trial | Media | Mean $C_A^{V'}$ | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 167 (0.0105) | 5 | 43 (0.0027) | A | 0.12 | 0.8855 |
| | 2 | 155 (0.0097) | 5 | 41 (0.0026) | A | | |
| | 3 | 156 (0.0098) | 5 | 47 (0.0029) | A | | |
| 2 | 1 | 228 (0.0142) | 5 | 33 (0.0021) | A | 15.46 | 0.0005 |
| | 2 | 176 (0.0110) | 5 | 20 (0.0013) | B | | |
| | 3 | 148 (0.0092) | 5 | 9 (0.0006) | B | | |
| 3 | 1 | 247 (0.0154) | 5 | 13 (0.0008) | A | 81.99 | <0.0001 |
| | 2 | 174 (0.0109) | 5 | 3 (0.0002) | B | | |
| | 3 | 187 (0.0117) | 5 | 10 (0.0006) | B | | |
| 4 | 1 | 217 (0.0135) | 6 | 47 (0.0030) | A | 3.99 | 0.0409 |
| | 2 | 162 (0.0101) | 6 | 21 (0.0013) | A | | |
| | 3 | 160 (0.0100) | 6 | 45 (0.0028) | A | | |
| 5 | 1 | 254 (0.0159) | 6 | 29 (0.0018) | A | 11.26 | 0.001 |
| | 2 | 187 (0.0117) | 6 | 32 (0.0020) | B | | |
| | 3 | 185 (0.0115) | 6 | 25 (0.0016) | B | | |

Table C.1-2. Comparison of volumetric nitratation rates ($C_N^{V'}$) normalized for influent TAN and $NO_2^-$-N as g $NO_2^-$-N/day-m$^3$ (lb $NO_2^-$-N/day-ft$^3$) of media systems by trial.

| Trial | Media | Mean $C_N^{V'}$ | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 182 (0.0114) | 5 | 139 (0.0087) | A | 0.15 | 0.8598 |
| | 2 | 160 (0.0100) | 5 | 110 (0.0069) | A | | |
| | 3 | 161 (0.0100) | 5 | 86 (0.0054) | A | | |
| 2 | 1 | 347 (0.0216) | 5 | 136 (0.0085) | A | 18.26 | 0.0002 |
| | 2 | 171 (0.0106) | 5 | 49 (0.0031) | B | | |
| | 3 | 169 (0.0106) | 5 | 31 (0.0020) | B | | |
| 3 | 1 | 279 (0.0174) | 5 | 27 (0.0017) | A | 42.14 | <0.0001 |
| | 2 | 174 (0.0108) | 5 | 19 (0.0012) | C | | |
| | 3 | 207 (0.0129) | 5 | 16 (0.0010) | B | | |
| 4 | 1 | 241 (0.0150) | 6 | 57 (0.0036) | A | 3.59 | 0.0533 |
| | 2 | 173 (0.0108) | 6 | 40 (0.0025) | B | | |
| | 3 | 195 (0.0121) | 6 | 57 (0.0035) | AB | | |
| 5 | 1 | 308 (0.0192) | 6 | 29 (0.0018) | A | 11.09 | 0.0011 |
| | 2 | 199 (0.0124) | 6 | 32 (0.0020) | B | | |
| | 3 | 228 (0.0142) | 6 | 25 (0.0016) | B | | |

Table C.1-3. Comparison of areal nitritation rates ( $C_A^{A'}$ ) normalized for influent TAN as mg TAN/day-$m^2$ (lb TAN/day-$ft^2$ x$10^{-5}$) of media systems by trial.

| Trial | Media | Mean $C_A^{A'}$ | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 153 (3.14) | 5 | 39 (0.80) | A | 0.59 | 0.5686 |
| | 2 | 134 (2.75) | 5 | 36 (0.73) | A | | |
| | 3 | 128 (2.63) | 5 | 38 (0.78) | A | | |
| 2 | 1 | 208 (4.26) | 5 | 30 (0.62) | A | 22.66 | <0.0001 |
| | 2 | 152 (3.11) | 5 | 17 (0.36) | B | | |
| | 3 | 121 (2.48) | 5 | 8 (0.16) | B | | |
| 3 | 1 | 225 (4.62) | 5 | 12 (0.24) | A | 127.22 | <0.0001 |
| | 2 | 151 (3.09) | 5 | 3 (0.60) | B | | |
| | 3 | 154 (3.14) | 5 | 8 (0.17) | B | | |
| 4 | 1 | 198 (4.06) | 6 | 43 (0.89) | A | 6.67 | 0.0085 |
| | 2 | 140 (2.87) | 6 | 18 (0.37) | B | | |
| | 3 | 131 (2.69) | 6 | 37 (0.76) | B | | |
| 5 | 1 | 232 (4.76) | 6 | 27 (0.55) | A | 18.36 | <0.0001 |
| | 2 | 162 (3.31) | 6 | 28 (0.57) | B | | |
| | 3 | 152 (3.11) | 6 | 21 (0.42) | B | | |

Table C.1-4. Comparison of areal nitratation rates ( $C_N^{A'}$ ) normalized for influent TAN and $NO_2^-$-N as mg $NO_2^-$-N/day-$m^2$ (lb $NO_2^-$-N/day-$ft^2$ x$10^{-5}$) of media systems by COD load trial grouping.

| Trial | Media | Mean $C_N^{A'}$ | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 166 (3.40) | 5 | 127 (2.60) | A | 0.42 | 0.6683 |
| | 2 | 138 (2.83) | 5 | 95 (1.95) | A | | |
| | 3 | 132 (2.70) | 5 | 71 (1.45) | A | | |
| 2 | 1 | 317 (6.49) | 5 | 124 (2.54) | A | 21.52 | 0.0001 |
| | 2 | 148 (3.02) | 5 | 43 (0.87) | B | | |
| | 3 | 139 (2.84) | 5 | 26 (0.53) | B | | |
| 3 | 1 | 255 (5.23) | 5 | 25 (0.51) | A | 56.04 | <0.0001 |
| | 2 | 150 (3.08) | 5 | 16 (0.33) | B | | |
| | 3 | 169 (3.47) | 5 | 13 (0.27) | B | | |
| 4 | 1 | 220 (4.51) | 6 | 52 (1.07) | A | 5.71 | 0.0144 |
| | 2 | 150 (3.07) | 6 | 35 (0.71) | B | | |
| | 3 | 160 (3.27) | 6 | 47(0.95) | B | | |
| 5 | 1 | 282 (5.77) | 6 | 27 (0.55) | A | 16.43 | 0.0002 |
| | 2 | 172 (3.53) | 6 | 28 (0.57) | B | | |
| | 3 | 187 (3.83) | 6 | 21 (0.42) | B | | |

## C.2 Nitrification Rates of Trials by Media System

Table C.2-1. Comparison of volumetric nitritation rates ($C_A^{V'}$) normalized for influent TAN as g TAN/day-m$^3$ (lb TAN/day-ft$^3$) of trials by media system.

| Media | Trial | Mean $C_A^{V'}$ | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 167 (0.0105) | 5 | 43 (0.0027) | B | 4.83 | 0.006 |
| | 2 | 228 (0.0142) | 5 | 33 (0.0021) | AB | | |
| | 3 | 247 (0.0154) | 5 | 13 (0.0008) | A | | |
| | 4 | 217 (0.0135) | 6 | 47 (0.0030) | AB | | |
| | 5 | 254 (0.0159) | 6 | 29 (0.0018) | A | | |
| 2 | 1 | 155 (0.0097) | 5 | 41 (0.0026) | A | 1.18 | 0.3454 |
| | 2 | 176 (0.0110) | 5 | 20 (0.0013) | A | | |
| | 3 | 174 (0.0109) | 5 | 3 (0.0002) | A | | |
| | 4 | 162 (0.0101) | 6 | 21 (0.0013) | A | | |
| | 5 | 187 (0.0117) | 6 | 32 (0.0020) | A | | |
| 3 | 1 | 156 (0.0098) | 5 | 47 (0.0029) | A | 1.61 | 0.2077 |
| | 2 | 148 (0.0092) | 5 | 9 (0.0006) | A | | |
| | 3 | 187 (0.0117) | 5 | 10 (0.0006) | A | | |
| | 4 | 160 (0.0100) | 6 | 45 (0.0028) | A | | |
| | 5 | 185 (0.0115) | 6 | 25 (0.0016) | A | | |

Table C.2-2. Comparison of volumetric nitratation rates ($C_N^{V'}$) normalized for influent TAN and $NO_2^-$-N as g $NO_2^-$-N/day-m$^3$ (lb $NO_2^-$-N/day-ft$^3$) of trials by media system.

| Media | Trial | Mean $C_N^{V'}$ | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 182 (0.0114) | 5 | 139 (0.0087) | B | 5.41 | 0.0035 |
| | 2 | 347 (0.0216) | 5 | 136 (0.0085) | A | | |
| | 3 | 279 (0.0174) | 5 | 27 (0.0017) | AB | | |
| | 4 | 241 (0.0150) | 6 | 57 (0.0036) | AB | | |
| | 5 | 308 (0.0192) | 6 | 29 (0.0018) | A | | |
| 2 | 1 | 160 (0.0100) | 5 | 110 (0.0069) | A | 0.58 | 0.6776 |
| | 2 | 171 (0.0106) | 5 | 49 (0.0031) | A | | |
| | 3 | 174 (0.0108) | 5 | 19 (0.0012) | A | | |
| | 4 | 173 (0.0108) | 6 | 40 (0.0025) | A | | |
| | 5 | 199 (0.0124) | 6 | 32 (0.0020) | A | | |
| 3 | 1 | 161 (0.0100) | 5 | 86 (0.0054) | B | 3.33 | 0.0282 |
| | 2 | 169 (0.0106) | 5 | 31 (0.0020) | AB | | |
| | 3 | 207 (0.0129) | 5 | 16 (0.0010) | AB | | |
| | 4 | 195 (0.0121) | 6 | 57 (0.0035) | AB | | |
| | 5 | 228 (0.0142) | 6 | 25 (0.0016) | A | | |

Table C.2-3. Comparison of areal nitritation rates ( $C_A^{A'}$ ) normalized for influent TAN as mg TAN/day-$m^2$ (lb TAN/day-$ft^2$ x$10^{-5}$) of trials by media system.

| Media | Trial | Mean $C_A^{A'}$ | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 153 (3.14) | 5 | 39 (0.80) | B | 4.83 | 0.0060 |
| | 2 | 208 (4.26) | 5 | 30 (0.62) | AB | | |
| | 3 | 225 (4.62) | 5 | 12 (0.24) | A | | |
| | 4 | 198 (4.06) | 6 | 43 (0.89) | AB | | |
| | 5 | 232 (4.76) | 6 | 27 (0.55) | A | | |
| 2 | 1 | 134 (2.75) | 5 | 36 (0.73) | A | 1.18 | 0.3454 |
| | 2 | 152 (3.11) | 5 | 17 (0.36) | A | | |
| | 3 | 151 (3.09) | 5 | 3 (0.60) | A | | |
| | 4 | 140 (2.87) | 6 | 18 (0.37) | A | | |
| | 5 | 162 (3.31) | 6 | 28 (0.57) | A | | |
| 3 | 1 | 128 (2.63) | 5 | 38 (0.78) | A | 1.61 | 0.2077 |
| | 2 | 121 (2.48) | 5 | 8 (0.16) | A | | |
| | 3 | 154 (3.14) | 5 | 8 (0.17) | A | | |
| | 4 | 131 (2.69) | 6 | 37 (0.76) | A | | |
| | 5 | 152 (3.11) | 6 | 21 (0.42) | A | | |

Table C.2-4. Comparison of areal nitratation rates ( $C_N^{A'}$ ) normalized for influent TAN and $NO_2^-$-N as mg $NO_2^-$-N/day-$m^2$ (lb $NO_2^-$-N/day-$ft^2$ x$10^{-5}$) of trials by media system.

| Media | Trial | Mean $C_N^{A'}$ | N | Std Dev | Tukey Grouping | F | p |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 166 (3.40) | 5 | 127 (2.60) | B | 5.41 | 0.0035 |
| | 2 | 317 (6.49) | 5 | 124 (2.54) | A | | |
| | 3 | 255 (5.23) | 5 | 25 (0.51) | AB | | |
| | 4 | 220 (4.51) | 6 | 52 (1.07) | AB | | |
| | 5 | 282 (5.77) | 6 | 27 (0.55) | A | | |
| 2 | 1 | 138 (2.83) | 5 | 95 (1.95) | A | 0.58 | 0.6776 |
| | 2 | 148 (3.02) | 5 | 43 (0.87) | A | | |
| | 3 | 150 (3.08) | 5 | 16 (0.33) | A | | |
| | 4 | 150 (3.07) | 6 | 35 (0.71) | A | | |
| | 5 | 172 (3.53) | 6 | 28 (0.57) | A | | |
| 3 | 1 | 132 (2.70) | 5 | 71 (1.45) | B | 3.33 | 0.282 |
| | 2 | 139 (2.84) | 5 | 26 (0.53) | AB | | |
| | 3 | 169 (3.47) | 5 | 13 (0.27) | AB | | |
| | 4 | 160 (3.27) | 6 | 47(0.95) | AB | | |
| | 5 | 187 (3.83) | 6 | 21 (0.42) | A | | |

## APPENDIX D.

## MEDIA DIMENSIONAL MEASUREMENTS

Table D-1. Media dimensional measurements (1/10 inch).

| Count | Media 1 | | | Media 2 | | | Media 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | D1 | D2 | H | D1 | D2 | H | D1 | D2 | H |
| 1 | 1.72 | 1.7 | 1.4 | 1.75 | 1.47 | 1.33 | 2.54 | 1.85 | 1.27 |
| 2 | 1.8 | 1.73 | 0.89 | 1.85 | 1.39 | 1.32 | 1.41 | 1.12 | 1.17 |
| 3 | 1.8 | 1.74 | 1.14 | 1.64 | 1.45 | 1.34 | 1.34 | 1.05 | 1.26 |
| 4 | 2 | 1.91 | 0.65 | 1.42 | 1.34 | 0.91 | 1.91 | 1.71 | 1.31 |
| 5 | 2 | 1.93 | 1.22 | 1.73 | 1.49 | 1.32 | 1.52 | 1.22 | 1.37 |
| 6 | 1.94 | 1.93 | 1.07 | 1.62 | 1.44 | 1.3 | 1.31 | 1.28 | 1.26 |
| 7 | 1.71 | 1.58 | 1.39 | 1.45 | 1.32 | 1.04 | 1.52 | 1.39 | 1.49 |
| 8 | 1.71 | 1.71 | 1.2 | 1.69 | 1.47 | 1.22 | 2.34 | 1.95 | 1.26 |
| 9 | 1.91 | 1.76 | 1.75 | 1.56 | 1.33 | 1.07 | 1.47 | 1.07 | 1.21 |
| 10 | 1.78 | 1.69 | 1.18 | 1.73 | 1.46 | 1.15 | 1.52 | 0.98 | 1.25 |
| 11 | 1.69 | 1.62 | 0.99 | 1.56 | 1.36 | 1.16 | 2.25 | 1.93 | 1.38 |
| 12 | 2.02 | 1.94 | 1.07 | 1.61 | 1.47 | 1.32 | 1.32 | 1.27 | 1.25 |
| 13 | 1.72 | 1.8 | 1.25 | 1.53 | 1.32 | 1.02 | 1.55 | 1.49 | 1.31 |
| 14 | 1.96 | 1.88 | 1.3 | 1.61 | 1.41 | 1.35 | 1.74 | 1.63 | 1.27 |
| 15 | 1.81 | 1.8 | 1.25 | 1.76 | 1.56 | 1.4 | 2.3 | 1.53 | 1.36 |
| 16 | 1.81 | 1.69 | 1.05 | 1.74 | 1.41 | 1.21 | 1.24 | 0.68 | 1.34 |
| 17 | 1.94 | 1.87 | 1.42 | 1.7 | 1.45 | 1.25 | 2.06 | 2.03 | 1.27 |
| 18 | 1.86 | 1.8 | 1.2 | 1.7 | 1.57 | 1.27 | 2.65 | 1.59 | 1.33 |
| 19 | 1.84 | 1.72 | 1.45 | 1.53 | 1.42 | 1.3 | 1.44 | 0.77 | 1.43 |
| 20 | 2.47 | 2.44 | 1.63 | 1.64 | 1.37 | 1.18 | 1.33 | 1.32 | 1.25 |
| 21 | 2.23 | 2.16 | 1.12 | 1.51 | 1.41 | 1.05 | 1.76 | 1.25 | 1.49 |
| 22 | 1.77 | 1.75 | 1.07 | 1.89 | 1.56 | 1.46 | 1.75 | 1.52 | 1.35 |
| 23 | 1.79 | 1.58 | 0.98 | 1.64 | 1.35 | 1.17 | 2.24 | 0.99 | 1.32 |
| 24 | 1.78 | 1.75 | 1.35 | 1.73 | 1.63 | 1.46 | 1.56 | 1.01 | 1.27 |
| 25 | 1.85 | 1.78 | 1.2 | 1.6 | 1.4 | 1.19 | 1.56 | 0.95 | 1.31 |
| 26 | 1.66 | 1.59 | 0.99 | 1.72 | 1.54 | 1.14 | 1.35 | 1.29 | 1.37 |
| 27 | 1.86 | 1.71 | 1.28 | 1.65 | 1.38 | 1.14 | 2.38 | 1.25 | 1.33 |
| 28 | 1.72 | 1.72 | 1.32 | 1.69 | 1.32 | 1.25 | 1.45 | 1.31 | 1.28 |
| 29 | 2.26 | 2.13 | 1.4 | 1.64 | 1.48 | 1.13 | 2.17 | 1.39 | 1.38 |
| 30 | 2.19 | 2.17 | 1.07 | 1.6 | 1.47 | 1.35 | 1.66 | 1 | 1.41 |
| 31 | 1.81 | 1.78 | 1.22 | 1.73 | 1.4 | 1.19 | 2.75 | 1.63 | 1.35 |
| 32 | 1.87 | 1.75 | 1.28 | 1.62 | 1.42 | 1.26 | 1.99 | 1.7 | 1.19 |
| 33 | 1.69 | 1.52 | 0.9 | 1.65 | 1.49 | 1.27 | 1.74 | 1.6 | 1.38 |
| 34 | 2 | 1.97 | 1.67 | 1.7 | 1.4 | 1.18 | 1.48 | 1.14 | 1.29 |
| 35 | 1.75 | 1.65 | 1.09 | 1.63 | 1.4 | 1.4 | 2.63 | 1.39 | 1.21 |
| 36 | 1.78 | 1.62 | 1.02 | 1.6 | 1.39 | 1.08 | 1.47 | 1.46 | 1.3 |
| 37 | 1.75 | 1.68 | 0.78 | 1.69 | 1.52 | 1.38 | 1.08 | 1.04 | 1.35 |

Table D-1. Continued.

| | Media 1 | | | Media 2 | | | Media 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| Count | D1 | D2 | H | D1 | D2 | H | D1 | D2 | H |
| 38 | 1.99 | 1.86 | 1.01 | 1.65 | 1.48 | 1.35 | 1.05 | 1.01 | 1.34 |
| 39 | 2.01 | 2.01 | 1.01 | 1.64 | 1.46 | 1.24 | 1.3 | 1.03 | 1.32 |
| 40 | 1.88 | 1.7 | 0.76 | 1.55 | 1.37 | 1.12 | 1.97 | 1.34 | 1.31 |
| 41 | 1.72 | 1.8 | 1.38 | 1.66 | 1.49 | 1.2 | 1.37 | 1.36 | 1.32 |
| 42 | 1.88 | 1.85 | 1.06 | 1.66 | 1.54 | 1.25 | 1.62 | 1.4 | 1.32 |
| 43 | 1.88 | 1.76 | 1.4 | 1.81 | 1.48 | 1.33 | 1.47 | 0.76 | 1.39 |
| 44 | 1.88 | 1.8 | 1.19 | 1.66 | 1.54 | 1.3 | 1.34 | 1.27 | 1.29 |
| 45 | 1.74 | 1.73 | 1.01 | 1.65 | 1.48 | 1.15 | 2.23 | 1.34 | 1.24 |
| 46 | 1.79 | 1.7 | 1.08 | 1.69 | 1.38 | 1.23 | 2.32 | 1.65 | 1.4 |
| 47 | 1.81 | 1.71 | 1.13 | 1.66 | 1.42 | 1.31 | 2.06 | 1.28 | 1.22 |
| 48 | 1.79 | 1.76 | 1.08 | 1.77 | 1.47 | 1.29 | 2.36 | 1.66 | 1.36 |
| 49 | 1.73 | 1.6 | 0.9 | 1.65 | 1.45 | 1.27 | 2.42 | 1.39 | 1.21 |
| 50 | 1.59 | 1.58 | 0.92 | 1.82 | 1.45 | 1.42 | 1.72 | 1.42 | 1.58 |
| 51 | 1.77 | 1.67 | 1.13 | 1.61 | 1.51 | 1.34 | 1.54 | 1.31 | 1.3 |
| 52 | 1.83 | 1.86 | 1.45 | 1.77 | 1.37 | 1.28 | 1.94 | 1.48 | 1.43 |
| 53 | 1.86 | 1.85 | 1.26 | 1.68 | 1.5 | 1.19 | 1.31 | 1.09 | 1.29 |
| 54 | 1.82 | 1.8 | 1.28 | 1.82 | 1.49 | 1.13 | 1.35 | 1.32 | 1.28 |
| 55 | 1.87 | 1.78 | 1.24 | 1.85 | 1.61 | 1.32 | 2.2 | 1.46 | 1.29 |
| 56 | 1.78 | 1.7 | 1.2 | 1.67 | 1.37 | 1.09 | 1.94 | 1.89 | 1.37 |
| 57 | 1.81 | 1.69 | 1.04 | 1.68 | 1.49 | 1.21 | 2.75 | 1.78 | 1.33 |
| 58 | 2.08 | 1.95 | 0.8 | 1.68 | 1.37 | 1.27 | 2.3 | 1.36 | 1.37 |
| 59 | 1.89 | 1.79 | 1.18 | 1.57 | 1.43 | 1.11 | 1.76 | 1.15 | 1.45 |
| 60 | 1.78 | 1.68 | 1.16 | 1.62 | 1.26 | 1.08 | 1.97 | 1.17 | 1.35 |
| 61 | 2.14 | 2.01 | 1.66 | 1.65 | 1.38 | 1.05 | 1.54 | 1.01 | 1.34 |
| 62 | 1.86 | 1.73 | 1.38 | 1.69 | 1.49 | 1.57 | 1.61 | 0.94 | 1.32 |
| 63 | 1.8 | 1.74 | 1.03 | 1.64 | 1.49 | 1.22 | 2.1 | 1.38 | 1.29 |
| 64 | 1.65 | 1.61 | 0.93 | 1.65 | 1.34 | 1.21 | 1.7 | 1.38 | 1.18 |
| 65 | 1.75 | 1.72 | 1.25 | 1.62 | 1.46 | 1.27 | 1.26 | 1.18 | 1.24 |
| 66 | 1.71 | 1.65 | 1.3 | 1.51 | 1.39 | 1.1 | 1.51 | 1.78 | 1.23 |
| 67 | 1.67 | 1.62 | 0.88 | 1.72 | 1.38 | 1.56 | 1.97 | 1.32 | 1.44 |
| 68 | 1.7 | 1.47 | 0.92 | 1.58 | 1.36 | 1.17 | 1.34 | 1.24 | 1.3 |
| 69 | 1.77 | 1.75 | 1.15 | 1.71 | 1.56 | 1.37 | 1.19 | 1.16 | 1.21 |
| 70 | 1.8 | 1.77 | 1.04 | 1.62 | 1.42 | 1.17 | 1.62 | 1.46 | 1.42 |
| 71 | 1.8 | 1.79 | 1.16 | 1.58 | 1.24 | 1.4 | 2 | 1.97 | 1.37 |
| 72 | 1.85 | 1.78 | 0.93 | 1.8 | 1.54 | 1.53 | 1.65 | 1.13 | 1.18 |
| 73 | 1.74 | 1.68 | 1.17 | 1.54 | 1.4 | 1.12 | 1.18 | 1.1 | 1.31 |
| 74 | 1.82 | 1.76 | 1.05 | 1.48 | 1.35 | 1.1 | 1.79 | 0.97 | 1.35 |
| 75 | 2.01 | 1.91 | 0.92 | 1.7 | 1.46 | 1.33 | 2.25 | 1.98 | 1.35 |
| 76 | 1.74 | 1.68 | 1.06 | 1.54 | 1.38 | 0.99 | 1 | 0.97 | 1.17 |
| 77 | 1.78 | 1.72 | 1.3 | 1.62 | 1.45 | 1.13 | 2.46 | 1.47 | 1.34 |
| 78 | 1.76 | 1.68 | 1.17 | 1.73 | 1.48 | 1.33 | 1.41 | 1.48 | 1.29 |

Table D-1. Continued.

| | Media 1 | | | Media 2 | | | Media 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| Count | D1 | D2 | H | D1 | D2 | H | D1 | D2 | H |
| 79 | 2.03 | 1.87 | 1.78 | 1.68 | 1.43 | 1.3 | 2.51 | 1.25 | 1.15 |
| 80 | 1.7 | 1.68 | 0.94 | 1.59 | 1.46 | 1.2 | 1.68 | 1.23 | 1.17 |
| 81 | 1.77 | 1.76 | 1.28 | 1.6 | 1.41 | 1.35 | 1.3 | 0.98 | 1.17 |
| 82 | 1.92 | 1.89 | 1.64 | 1.77 | 1.46 | 1.12 | 2.15 | 1.81 | 2.61 |
| 83 | 1.91 | 1.9 | 1.59 | 1.6 | 1.39 | 1.18 | 2.5 | 1.63 | 1.27 |
| 84 | 1.77 | 1.67 | 1.42 | 1.67 | 1.39 | 1.21 | 1.93 | 1.84 | 1.23 |
| 85 | 1.63 | 1.49 | 0.76 | 1.8 | 1.43 | 1.56 | 1.71 | 1.19 | 1.26 |
| 86 | 1.89 | 1.79 | 1.23 | 1.84 | 1.62 | 1.39 | 2.06 | 0.98 | 1.52 |
| 87 | 1.7 | 1.64 | 1.2 | 1.63 | 1.46 | 1.38 | 2.57 | 1.92 | 1.3 |
| 88 | 1.95 | 1.87 | 0.91 | 1.65 | 1.49 | 1.41 | 2.05 | 1.46 | 1.27 |
| 89 | 1.85 | 1.73 | 1.17 | 1.75 | 1.43 | 1.17 | 1.18 | 1.02 | 1.24 |
| 90 | 1.96 | 1.82 | 1.01 | 1.71 | 1.47 | 1.3 | 1.52 | 0.89 | 1.3 |
| 91 | 1.89 | 1.82 | 0.98 | 1.71 | 1.53 | 1.15 | 1.28 | 1.21 | 1.2 |
| 92 | 1.9 | 1.81 | 1.04 | 1.67 | 1.52 | 1.37 | 2.41 | 2.1 | 1.18 |
| 93 | 2.23 | 2.22 | 1.24 | 1.62 | 1.44 | 1.37 | 2.14 | 1.36 | 1.25 |
| 94 | 1.72 | 1.61 | 0.98 | 1.63 | 1.49 | 1.14 | 1.35 | 1.23 | 1.24 |
| 95 | 1.61 | 1.57 | 1.05 | 1.67 | 1.5 | 1.33 | 1.5 | 1.35 | 1.25 |
| 96 | 2.06 | 1.84 | 0.47 | 1.63 | 1.46 | 1.41 | 1.8 | 1.49 | 1.29 |
| 97 | 1.84 | 1.84 | 1.27 | 1.72 | 1.38 | 1.37 | 1.76 | 1.04 | 1.38 |
| 98 | 0.91 | 0.79 | 0.21 | 1.62 | 1.45 | 1.23 | 2.41 | 1.72 | 1.32 |
| 99 | 1.67 | 1.63 | 0.85 | 1.52 | 1.33 | 1.09 | 1.64 | 1.08 | 1.42 |
| 100 | 1.77 | 1.74 | 1.1 | 1.61 | 1.4 | 1.4 | 2.69 | 1.55 | 1.28 |

## APPENDIX E.

## RAW WATER QUALITY DATA

Table E1. Steady-state water quality data.

| Run | Sys | Date | Alk (mg/L as $CaCO_3$) | feed (g) | TAN (mg/L) | | $NO_2^-$-N (mg/L) | | $NO_3^-$-N (mg/L) | | Temp (°C) | DOccmb (mg/L) | | DOtank (mg/L) | pH | $Na_2CO_3$ (g) | COD (mg/L) | | TN (mg/L) | | Flush (gal) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | In | Out | In | Out | In | Out | | In | Out | | | | In | Out | In | Out | |
| 1 | 1 | 2/28/98 | 156 | 400 | 1.021 | 0.690 | 0.051 | 0.048 | 14.4 | 14.4 | 25.2 | 4.75 | 5.61 | 5.81 | 7.43 | | 21 | 21 | 25.8 | 25.5 | 77.75 |
| 1 | 2 | 2/28/98 | 158 | 400 | 0.928 | 0.690 | 0.085 | 0.074 | 15.3 | 15.4 | 25.5 | 4.76 | 5.07 | 5.87 | 7.47 | | 13 | 13 | 23.3 | 20.4 | 77.75 |
| 1 | 3 | 2/28/98 | 158 | 400 | 1.257 | 0.860 | 0.065 | 0.069 | 14.6 | 14.5 | 24.6 | 4.74 | 5.03 | 5.79 | 7.46 | | 15 | 15 | 13.8 | 13.8 | 77.75 |
| 1 | 1 | 3/1/98 | 143 | 400 | 0.835 | 0.596 | 0.049 | 0.060 | 13.3 | 11.7 | 24.7 | 4.78 | 5.07 | 5.75 | 7.39 | 200 | 25 | 26 | 35.8 | 37.5 | 77.75 |
| 1 | 2 | 3/1/98 | 152 | 400 | 0.759 | 0.579 | 0.106 | 0.095 | 13.8 | 12.3 | 25.0 | 4.99 | 5.32 | 6.02 | 7.43 | 100 | 20 | 22 | 23.4 | 26.2 | 77.75 |
| 1 | 3 | 3/1/98 | 148 | 400 | 0.835 | 0.639 | 0.073 | 0.075 | 12.0 | 11.4 | 23.9 | 5.13 | 5.30 | 6.03 | 7.42 | 100 | 12 | 11 | 28.6 | 31.4 | 77.75 |
| 1 | 1 | 3/2/98 | 173 | 400 | 0.639 | 0.493 | 0.078 | 0.068 | 15.4 | 15.5 | 24.1 | 4.75 | 4.99 | 5.81 | 7.53 | | 22 | 18 | 36.4 | 35.9 | 77.75 |
| 1 | 2 | 3/2/98 | 148 | 400 | 0.433 | 0.364 | 0.118 | 0.129 | 15.2 | 15.8 | 24.5 | 5.04 | 5.34 | 6.22 | 7.51 | 50 | 30 | 24 | 36.3 | 34.8 | 77.75 |
| 1 | 3 | 3/2/98 | 156 | 400 | 0.320 | 0.277 | 0.092 | 0.085 | 15.2 | 15.0 | 23.4 | 5.23 | 5.40 | 6.21 | 7.49 | 50 | 20 | 15 | 30.7 | 25.6 | 77.75 |
| 1 | 1 | 3/3/98 | 173 | 400 | 0.690 | 0.511 | 0.043 | 0.042 | 15.2 | 16.5 | 25.3 | 5.05 | 5.43 | 6.13 | 7.56 | | 21 | 22 | 16.3 | 55.1 | 77.75 |
| 1 | 2 | 3/3/98 | 164 | 400 | 0.699 | 0.476 | 0.086 | 0.092 | 16.4 | 15.9 | 25.3 | 5.26 | 5.56 | 6.38 | 7.58 | | 31 | 20 | 60.9 | 51.3 | 77.75 |
| 1 | 3 | 3/3/98 | 154 | 400 | 0.733 | 0.528 | 0.063 | 0.064 | 15.2 | 15.4 | 24.6 | 5.33 | 5.47 | 6.20 | 7.56 | 100 | 25 | 6 | 93.5 | 53.2 | 77.75 |
| 1 | 1 | 3/4/98 | 162 | 400 | 0.502 | 0.424 | 0.074 | 0.065 | 16.9 | 15.4 | 26.7 | 4.04 | 4.45 | 5.27 | 7.46 | 50 | 25 | 18 | 14.6 | 16.6 | 77.75 |
| 1 | 2 | 3/4/98 | 157 | 400 | 0.545 | 0.442 | 0.144 | 0.149 | 16.8 | 16.7 | 26.7 | 4.4 | 4.68 | 5.62 | 7.49 | 50 | 27 | 19 | 22.9 | 26.3 | 77.75 |
| 1 | 3 | 3/4/98 | 173 | 400 | 0.707 | 0.562 | 0.113 | 0.106 | 16.0 | 16.8 | 26.1 | 4.29 | 4.39 | 5.35 | 7.48 | | 4 | 12 | 18.8 | 15.1 | 77.75 |
| 2 | 1 | 3/22/98 | 156 | 1000 | 0.648 | 0.422 | 0.348 | 0.284 | 35.0 | 35.4 | 26.4 | 3.63 | 3.42 | 5.67 | 7.45 | 100 | 88 | 79 | 28.1 | 31.4 | 71.75 |
| 2 | 2 | 3/22/98 | 171 | 1000 | 0.690 | 0.510 | 0.699 | 0.673 | 37.5 | 38.5 | 26.1 | 3.80 | 3.74 | 5.70 | 7.51 | 50 | 87 | 76 | 28.3 | 38.9 | 71.75 |
| 2 | 3 | 3/22/98 | 164 | 1000 | 0.724 | 0.564 | 1.237 | 1.200 | 38.4 | 40.5 | 25.6 | 3.34 | 3.31 | 5.30 | 7.45 | 75 | 82 | 76 | 40.5 | 37.1 | 71.75 |
| 2 | 1 | 3/23/98 | 161 | 1000 | 0.852 | 0.536 | 1.218 | 1.198 | 34.2 | 34.9 | 26.1 | 3.42 | 3.26 | 5.72 | 7.48 | 75 | 104 | 102 | 35.5 | 53.6 | 71.75 |
| 2 | 2 | 3/23/98 | 174 | 1000 | 0.733 | 0.562 | 1.152 | 1.118 | 37.6 | 38.3 | 26.5 | 3.76 | 3.69 | 5.75 | 7.5 | 50 | 86 | 92 | 33.3 | 38.8 | 71.75 |
| 2 | 3 | 3/23/98 | 163 | 1000 | 0.733 | 0.569 | 1.208 | 1.170 | 38.7 | 40.4 | 25.4 | 3.21 | 3.32 | 5.36 | 7.46 | 75 | 89 | 84 | 45.4 | 40.5 | 71.75 |
| 2 | 1 | 3/24/98 | 170 | 1000 | 1.097 | 0.751 | 0.265 | 0.243 | 31.6 | 32.9 | 24.9 | 4.72 | 4.30 | 6.33 | 7.48 | 50 | 97 | 96 | 64.1 | 67.6 | 71.75 |

Table E1 Continued.

| Run | Sys | Date | Alk (mg/L as $CaCO_3$) | feed (g) | TAN (mg/L) | | $NO_2^-$-N (mg/L) | | $NO_3^-$-N (mg/L) | | Temp (°C) | DOccmb (mg/L) | | DOtank (mg/L) | pH | $Na_2CO_3$ (g) | COD (mg/L) | | TN (mg/L) | | Flush (gal) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | In | Out | In | Out | In | Out | | In | Out | | | | In | Out | In | Out | |
| 2 | 2 | 3/24/98 | 169 | 1000 | 1.257 | 0.875 | 0.467 | 0.495 | 31.4 | 31.8 | 25.3 | 3.4 | 3.34 | 5.67 | 7.52 | 75 | 88 | 93 | 60.9 | 39.1 | 71.75 |
| 2 | 3 | 3/24/98 | 172 | 1000 | 1.484 | 1.165 | 0.847 | 0.855 | 33.5 | 35.8 | 24.5 | 4.67 | 4.18 | 6.29 | 7.48 | 50 | 104 | 103 | 42.8 | 47.4 | 71.75 |
| 2 | 1 | 3/25/98 | 165 | 1000 | 0.181 | 0.132 | 0.375 | 0.217 | 31.3 | 33.6 | 25.3 | 4.08 | 3.9 | 6.22 | 7.49 | 75 | 105 | 97 | 40.3 | 48.1 | 71.75 |
| 2 | 2 | 3/25/98 | 175 | 1000 | 0.233 | 0.178 | 0.445 | 0.465 | 31.3 | 31.8 | 25.6 | 4.29 | 4.03 | 5.90 | 7.54 | 50 | 78 | 91 | 31.4 | 34.6 | 71.75 |
| 2 | 3 | 3/25/98 | 170 | 1000 | 0.294 | 0.234 | 1.093 | 1.003 | 33.4 | 35.9 | 24.9 | 3.37 | 3.27 | 5.27 | 7.48 | 50 | 91 | 112 | 40.3 | 35.9 | 71.75 |
| 2 | 1 | 3/26/98 | 164 | 1000 | 0.416 | 0.250 | 0.347 | 0.283 | 38.6 | 37.9 | 25.9 | 3.43 | 3.36 | 5.69 | 7.44 | 75 | 91 | 87 | 54.6 | 46.4 | 71.75 |
| 2 | 2 | 3/26/98 | 173 | 1000 | 0.381 | 0.274 | 0.438 | 0.438 | 36.4 | 34.4 | 26.1 | 3.66 | 3.61 | 5.47 | 7.52 | 50 | 79 | 67 | 40.8 | 41.5 | 71.75 |
| 2 | 3 | 3/26/98 | 176 | 1000 | 0.511 | 0.387 | 1.533 | 1.493 | 39.8 | 39.1 | 25.6 | 3.18 | 3.01 | 5.03 | 7.47 | 50 | 79 | 83 | 47.9 | 44.3 | 71.75 |
| 3 | 1 | 4/4/98 | 165 | 1000 | 0.844 | 0.540 | 0.387 | 0.365 | 38.4 | 38.9 | 26.5 | 4.40 | 4.02 | 5.18 | 7.52 | 75 | 100 | 94 | 47.8 | 49.1 | 71.75 |
| 3 | 2 | 4/4/98 | 161 | 1000 | 0.827 | 0.613 | 0.677 | 0.657 | 38.4 | 37.5 | 26.7 | 4.80 | 4.38 | 5.36 | 7.55 | 100 | 82 | 88 | 44.6 | 41.6 | 71.75 |
| 3 | 3 | 4/4/98 | 160 | 1000 | 0.894 | 0.656 | 3.560 | 3.460 | 40.5 | 39.1 | 25.8 | 4.38 | 3.85 | 4.95 | 7.50 | 100 | 95 | 97 | 51.8 | 50.9 | 71.75 |
| 3 | 1 | 4/5/98 | 160 | 1000 | 0.920 | 0.569 | 0.410 | 0.388 | 43.1 | 43.4 | 26.2 | 4.40 | 3.76 | 5.25 | 7.51 | 100 | 109 | 112 | 54.6 | 60.6 | 71.75 |
| 3 | 2 | 4/5/98 | 160 | 1000 | 0.827 | 0.613 | 0.580 | 0.580 | 39.6 | 40.8 | 26.4 | 4.84 | 4.37 | 5.31 | 7.55 | 100 | 88 | 85 | 56.1 | 59.1 | 71.75 |
| 3 | 3 | 4/5/98 | 159 | 1000 | 0.937 | 0.657 | 3.447 | 3.347 | 42.4 | 42.4 | 25.2 | 4.55 | 3.89 | 5.20 | 7.50 | 100 | 86 | 83 | 61.0 | 61.6 | 71.75 |
| 3 | 1 | 4/6/98 | 165 | 1000 | 0.869 | 0.540 | 0.410 | 0.387 | 42.6 | 39.6 | 26.1 | 4.4 | 3.82 | 5.26 | 7.46 | 100 | 109 | 108 | 77.4 | 100.8 | 71.75 |
| 3 | 2 | 4/6/98 | 160 | 1000 | 0.827 | 0.613 | 0.610 | 0.615 | 40.6 | 37.7 | 26.3 | 4.78 | 4.35 | 5.36 | 7.50 | 150 | 86 | 86 | 78.9 | 70.5 | 71.75 |
| 3 | 3 | 4/6/98 | 159 | 1000 | 0.886 | 0.647 | 3.293 | 3.167 | 41.3 | 42.6 | 25.2 | 4.50 | 3.81 | 5.26 | 7.45 | 150 | 80 | 76 | 55.1 | 48.4 | 71.75 |
| 3 | 1 | 4/7/98 | 165 | 1000 | 0.971 | 0.641 | 0.460 | 0.455 | 41.9 | 41.4 | 26.6 | 4.03 | 3.46 | 4.66 | 7.47 | 165 | 93 | 101 | 63.6 | 59.4 | 71.75 |
| 3 | 2 | 4/7/98 | 173 | 1000 | 0.911 | 0.676 | 0.600 | 0.620 | 37.1 | 37.3 | 26.8 | 4.31 | 3.85 | 4.72 | 7.52 | 173 | 75 | 76 | 67.8 | 88.4 | 71.75 |
| 3 | 3 | 4/7/98 | 170 | 1000 | 1.004 | 0.730 | 3.273 | 3.160 | 41.4 | 41.8 | 25.9 | 4.01 | 3.32 | 4.62 | 3.46 | 170 | 86 | 85 | 53.6 | 76.5 | 71.75 |
| 3 | 1 | 4/8/98 | 160 | 1000 | 0.733 | 0.450 | 0.517 | 0.482 | 42.3 | 41.2 | 27.5 | 3.99 | 3.38 | 4.69 | 7.47 | 125 | 111 | 113 | 60.0 | 51.8 | 71.75 |
| 3 | 2 | 4/8/98 | 161 | 1000 | 0.656 | 0.479 | 0.602 | 0.598 | 40.1 | 38.3 | 27.6 | 4.31 | 3.90 | 4.83 | 7.50 | 125 | 78 | 85 | 41.8 | 47.3 | 71.75 |
| 3 | 3 | 4/8/98 | 162 | 1000 | 0.767 | 0.542 | 3.993 | 3.933 | 41.9 | 42.0 | 27.0 | 3.90 | 3.26 | 4.65 | 7.44 | 125 | 91 | 96 | 46.5 | 69.5 | 71.75 |
| 4 | 1 | 4/25/98 | 163 | 1000 | 0.685 | 0.467 | 0.767 | 0.736 | 42.9 | 45.0 | 26.5 | 5.00 | 4.71 | | 7.58 | 100 | 143 | 148 | 35.4 | 22.8 | 71.75 |
| 4 | 2 | 4/25/98 | 166 | 1000 | 0.719 | 0.529 | 0.436 | 0.447 | 39.1 | 40.9 | 26.5 | 5.25 | 4.84 | | 7.61 | 100 | 154 | 150 | 23.9 | 33.3 | 71.75 |
| 4 | 3 | 4/25/98 | 170 | 1000 | 0.650 | 0.504 | 0.786 | 0.729 | 36.7 | 37.5 | 25.8 | 4.95 | 4.64 | | 7.59 | 100 | 142 | 132 | 25.8 | 45.1 | 71.75 |
| 4 | 1 | +4 Hrs | | | 0.815 | 0.585 | 0.890 | 0.873 | 45.9 | 44.3 | 26.5 | 5.07 | 4.56 | | | | 155 | 159 | 47.1 | 55.0 | |

Table E1 Continued.

| Run | Sys | Date | Alk (mg/L as $CaCO_3$) | feed (g) | TAN (mg/L) | | $NO_2^-$-N (mg/L) | | $NO_3^-$-N (mg/L) | | Temp (°C) | DOccmb (mg/L) | | DOtank (mg/L) | pH | $Na_2CO_3$ (g) | COD (mg/L) | | TN (mg/L) | | Flush (gal) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | In | Out | In | Out | In | Out | | In | Out | | | | In | Out | In | Out | |
| 4 | 2 | +4 Hrs | | | 0.822 | 0.617 | 0.499 | 0.498 | 40.8 | 38.5 | 26.5 | 5.13 | 4.65 | | | | 160 | 169 | 35.5 | 13.4 | |
| 4 | 3 | +4 Hrs | | | 0.805 | 0.622 | 0.875 | 0.842 | 39.4 | 37.9 | 25.8 | 4.95 | 4.52 | | | | 160 | 159 | 37.6 | 28.5 | |
| 4 | 1 | +8 Hrs | | | 0.758 | 0.56 | 1.042 | 1.015 | 46.0 | 43.2 | 26.6 | 5.12 | 4.70 | | | | 143 | 151 | 47.8 | 40.0 | |
| 4 | 2 | +8 Hrs | | | 0.764 | 0.574 | 0.562 | 0.551 | 40.2 | 40.7 | 26.6 | 5.32 | 4.70 | | | | 150 | 140 | 33.8 | 29.0 | |
| 4 | 3 | +8 Hrs | | | 0.805 | 0.640 | 0.998 | 0.969 | 38.5 | 38.7 | 25.8 | 5.18 | 4.81 | | | | 147 | 136 | 34.3 | 20.8 | |
| 4 | 1 | +12 Hrs | | | 0.734 | 0.504 | 1.083 | 1.054 | 46.4 | 44.4 | 26.7 | 5.55 | 5.05 | | | | 144 | 149 | 40.5 | 30.9 | |
| 4 | 2 | +12 Hrs | | | 0.719 | 0.553 | 0.556 | 0.551 | 41.7 | 41.3 | 26.7 | 5.66 | 5.15 | | | | 151 | 150 | 14.0 | 31.3 | |
| 4 | 3 | +12 Hrs | | | 0.770 | 0.627 | 1.042 | 0.996 | 38.6 | 38.7 | 25.9 | 5.59 | 5.56 | | | | 137 | 135 | 60.9 | 44.8 | |
| 4 | 1 | +16 Hrs | | | 0.433 | 0.299 | 0.992 | 0.940 | 45.8 | 45.3 | 26.7 | 5.76 | 5.43 | | | | 143 | 147 | 62.0 | 61.6 | |
| 4 | 2 | +16 Hrs | | | 0.770 | 0.627 | 1.042 | 0.996 | 38.6 | 38.7 | 25.9 | 5.59 | 5.26 | | | | 150 | 150 | 64.9 | 53.6 | |
| 4 | 3 | +16 Hrs | | | 0.463 | 0.361 | 0.981 | 0.915 | 40.4 | 39.3 | 25.8 | 5.84 | 5.58 | | | | 156 | 151 | 54.1 | 55.0 | |
| 4 | 1 | +24 Hrs | | | 0.422 | 0.227 | 0.798 | 0.756 | 48.3 | 44.9 | 26.7 | 5.26 | 5.02 | | | | 141 | 145 | 71.5 | 71.1 | |
| 4 | 2 | +24 Hrs | | | 0.394 | 0.286 | 0.484 | 0.459 | 44.0 | 40.8 | 26.6 | 5.85 | 5.41 | | | | 154 | 162 | 92.3 | 65.3 | |
| 4 | 3 | +24 Hrs | | | 0.351 | 0.220 | 0.815 | 0.765 | 39.8 | 39.3 | 25.8 | 5.45 | 5.33 | | | | 156 | 137 | 61.4 | 51.8 | |
| 5 | 1 | 5/21/98 | 177 | 1000 | 0.627 | 0.481 | 0.386 | 0.363 | 45.7 | 44.2 | 26.6 | 5.2 | 5.23 | | 7.67 | 80 | 202 | 207 | 52.6 | 59.9 | 71.75 |
| 5 | 2 | 5/21/98 | 177 | 1000 | 0.776 | 0.627 | 0.413 | 0.404 | 43.7 | 43.4 | 26.4 | 5.57 | 5.51 | | 7.69 | 80 | 202 | 205 | 48.5 | 43.8 | 71.75 |
| 5 | 3 | 5/21/98 | 177 | 1000 | 0.595 | 0.488 | 0.333 | 0.329 | 41.5 | 40.8 | 25.9 | 5.92 | 5.99 | | 7.73 | 80 | 180 | 185 | 47.4 | 51.4 | 71.75 |
| 5 | 1 | +4 Hrs | | | 0.832 | 0.581 | 0.479 | 0.461 | 45.4 | 46.0 | 26.7 | 4.84 | 4.84 | | | | 207 | 202 | 61.1 | 58.4 | |
| 5 | 2 | +4 Hrs | | | 1.030 | 0.842 | 0.475 | 0.481 | 41.3 | 43.2 | 26.5 | 5.22 | 5.17 | | | | 212 | 185 | 59.4 | 57.8 | |
| 5 | 3 | +4 Hrs | | | 0.650 | 0.540 | 0.404 | 0.394 | 41.7 | 42.3 | 26.0 | 5.64 | 5.62 | | | | 170 | 170 | 61.8 | 58.1 | |
| 5 | 1 | +8 Hrs | | | 0.832 | 0.590 | 0.577 | 0.552 | 48.4 | 47.9 | 26.8 | 4.86 | 4.79 | | | | 200 | 213 | 67.6 | 50.1 | |
| 5 | 2 | +8 Hrs | | | 1.125 | 0.938 | 0.592 | 0.579 | 44.4 | 44.4 | 26.7 | 5.22 | 5.22 | | | | 199 | 197 | 42.3 | 62.3 | |
| 5 | 3 | +8 Hrs | | | 0.630 | 0.506 | 0.454 | 0.444 | 42.1 | 42.6 | 26.3 | 5.55 | 5.6 | | | | 186 | 184 | 45.1 | 44.5 | |
| 5 | 1 | +12 Hrs | | | 0.602 | 0.445 | 0.577 | 0.577 | 45.0 | 46.3 | 26.8 | 5.37 | 5.29 | | | | 214 | 203 | 42.3 | 44.9 | |
| 5 | 2 | +12 Hrs | | | 0.958 | 0.805 | 0.621 | 0.615 | 41.7 | 42.8 | 26.6 | 5.63 | 5.57 | | | | 191 | 195 | 43.6 | 41.6 | |
| 5 | 3 | +12 Hrs | | | 0.515 | 0.417 | 0.456 | 0.440 | 40.7 | 40.9 | 26.2 | 5.93 | 5.92 | | | | 175 | 163 | 40.8 | 36.3 | |

Table E1 Continued.

| Run | Sys | Date | Alk (mg/L as $CaCO_3$) | feed (g) | TAN (mg/L) | | $NO_2^-$-N (mg/L) | | $NO_3^-$-N (mg/L) | | Temp (°C) | DOccmb (mg/L) | | DOtank (mg/L) | pH | $Na_2CO_3$ (g) | COD (mg/L) | | TN (mg/L) | | Flush (gal) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | In | Out | In | Out | In | Out | | In | Out | | | | In | Out | In | Out | |
| 5 | 1 | +16 Hrs | | | 0.417 | 0.302 | 0.467 | 0.417 | 46.6 | 47.2 | 26.7 | 5.91 | 5.88 | | | | 220 | 199 | 52.1 | 72.0 | |
| 5 | 2 | +16 Hrs | | | 0.565 | 0.442 | 0.571 | 0.560 | 42.5 | 43.3 | 26.6 | 5.98 | 6.00 | | | | 211 | 199 | 55.8 | 60.1 | |
| 5 | 3 | +16 Hrs | | | 0.334 | 0.275 | 0.400 | 0.356 | 40.8 | 40.4 | 26.1 | 6.23 | 6.28 | | | | 165 | 169 | 67.5 | 38.3 | |
| 5 | 1 | +24 Hrs | | | 0.326 | 0.252 | 0.350 | 0.319 | 49.1 | 47.4 | 26.7 | 5.14 | 5.72 | | | | 194 | 203 | 59.9 | 64.1 | |
| 5 | 2 | +24 Hrs | | | 0.355 | 0.267 | 0.444 | 0.436 | 45.4 | 44.1 | 26.5 | 5.5 | 5.67 | | | | 950 | 670 | 72.3 | 58.3 | |
| 5 | 3 | +24 Hrs | | | 0.281 | 0.213 | 0.308 | 0.288 | 42.9 | 42.5 | 26.0 | 5.98 | 6.18 | | | | 950 | 670 | 72.3 | 58.3 | |

Table E2. Acclimation water quality data.

| System | Day | Date | Alk (mg/L as $CaCO_3$) | TAN (mg/L) | NO2-Nin (mg/L) | Temp (°C) | DO (Mg/L) | pH |
|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 1/5/98 | 150 | 12.68 | 5.20 | 27.4 | 7.60 | 8.20 |
| 2 | 1 | 1/5/98 | 135 | 12.89 | 5.15 | 27.3 | 7.50 | 8.18 |
| 3 | 1 | 1/5/98 | 150 | 13.00 | 5.10 | 27.3 | 7.60 | 8.27 |
| 1 | 2 | 1/6/98 | 140 | 8.49 | 5.50 | 34.0 | 6.60 | 8.27 |
| 2 | 2 | 1/6/98 | 123 | 9.37 | 5.25 | 32.6 | 7.00 | 8.32 |
| 3 | 2 | 1/6/98 | 145 | 7.65 | 5.05 | 32.3 | 7.00 | 8.35 |
| 1 | 3 | 1/7/98 | 355 | 1.82 | 5.30 | 30.1 | 7.02 | 8.92 |
| 2 | 3 | 1/7/98 | 340 | 2.88 | 5.25 | 28.0 | 7.31 | 8.93 |
| 3 | 3 | 1/7/98 | 350 | 3.14 | 5.05 | 27.7 | 7.52 | 8.83 |
| 1 | 4 | 1/8/98 | | 0.630 | 5.30 | 28.8 | 7.29 | 8.86 |
| 2 | 4 | 1/8/98 | | 1.20 | 5.10 | 28.4 | 7.40 | 8.93 |
| 3 | 4 | 1/8/98 | | 1.33 | 4.95 | 27.8 | 7.22 | 8.91 |
| 1 | 5 | 1/9/98 | | 0.189 | 5.45 | 29.5 | 7.42 | |
| 2 | 5 | 1/9/98 | | 0.497 | 4.95 | 28.6 | 7.43 | |
| 3 | 5 | 1/9/98 | | 0.673 | 4.75 | 27.9 | 7.24 | |
| 1 | 6 | 1/10/98 | | 0.077 | 5.45 | 29.3 | | |
| 2 | 6 | 1/10/98 | | 0.256 | 4.90 | 28.4 | | |
| 3 | 6 | 1/10/98 | | 0.390 | 4.75 | 27.6 | | |
| 1 | 7 | 1/11/98 | | 0.046 | 5.20 | 30.3 | | |
| 2 | 7 | 1/11/98 | | 0.165 | 4.55 | 29.5 | | |
| 3 | 7 | 1/11/98 | | 0.234 | 4.65 | 28.8 | | |
| 1 | 8 | 1/12/98 | | 0.033 | 5.05 | 29.0 | | |
| 2 | 8 | 1/12/98 | | 0.089 | 4.25 | 29.2 | | |
| 3 | 8 | 1/12/98 | | 0.103 | 4.75 | 28.6 | | |
| 1 | 9 | 1/13/98 | | 0.000 | 4.45 | 31.1 | | |
| 2 | 9 | 1/13/98 | | 0.000 | 3.85 | 30.8 | | |
| 3 | 9 | 1/13/98 | | 0.052 | 4.60 | 30.4 | | |
| 1 | 10 | 1/14/98 | | 0.000 | 4.15 | 29.3 | | |
| 2 | 10 | 1/14/98 | | 0.000 | 3.00 | 27.9 | | |
| 3 | 10 | 1/14/98 | | 0.000 | 4.60 | 27.5 | | |
| 1 | 11 | 1/15/98 | | | 3.50 | 32.5 | | |
| 2 | 11 | 1/15/98 | | | 1.75 | 31.7 | | |
| 3 | 11 | 1/15/98 | | | 4.55 | 31.1 | | |
| 1 | 12 | 1/16/98 | | | 2.45 | 29.5 | | |
| 2 | 12 | 1/16/98 | | | 0.450 | 28.8 | | |
| 3 | 12 | 1/16/98 | | | 4.45 | 28.4 | | |
| 1 | 13 | 1/17/98 | | | 1.65 | 30.5 | | 9.11 |
| 2 | 13 | 1/17/98 | | | 0.015 | 30.2 | | 9.11 |
| 3 | 13 | 1/17/98 | | | 4.35 | 29.7 | | 8.96 |

Table E2 Continued.

| System | Day | Date | Alk (mg/L as $CaCO_3$) | TAN (mg/L) | NO2-Nin (mg/L) | Temp (°C) | DO (Mg/L) | pH |
|---|---|---|---|---|---|---|---|---|
| 1 | 14 | 1/18/98 | | | 0.630 | | | |
| 2 | 14 | 1/18/98 | | | 0.015 | | | |
| 3 | 14 | 1/18/98 | | | 4.65 | | | |
| 1 | 15 | 1/19/98 | | | 0.200 | 28.2 | | |
| 2 | 15 | 1/19/98 | | | 0.038 | 27.1 | | |
| 3 | 15 | 1/19/98 | | | 4.55 | 26.2 | | |
| 1 | 16 | 1/20/98 | | | 0.060 | 34.7 | | |
| 2 | 16 | 1/20/98 | | | 0.052 | 33.9 | | |
| 3 | 16 | 1/20/98 | | | 4.80 | 33.6 | | |
| 1 | 17 | 1/21/98 | | | 0.002 | 29.5 | | |
| 2 | 17 | 1/21/98 | | | 0.004 | 27.5 | | |
| 3 | 17 | 1/21/98 | | | 3.85 | 27.4 | | |
| 1 | 18 | 1/22/98 | | | 0.004 | 29.5 | | |
| 2 | 18 | 1/22/98 | | | 0.000 | 27.5 | | |
| 3 | 18 | 1/22/98 | | | 2.75 | 27.6 | | |
| 1 | 19 | 1/23/98 | | | 0.000 | 31.8 | | |
| 2 | 19 | 1/23/98 | | | 0.000 | 30.5 | | |
| 3 | 19 | 1/23/98 | | | 1.060 | 30.4 | | |
| 1 | 20 | 1/24/98 | | | 0.000 | 31.8 | | |
| 2 | 20 | 1/24/98 | | | 0.000 | 30.1 | | |
| 3 | 20 | 1/24/98 | | | 0.120 | 30.1 | | |
| 1 | 21 | 1/25/98 | | | 0.000 | 33.9 | | |
| 2 | 21 | 1/25/98 | | | 0.000 | 32.9 | | |
| 3 | 21 | 1/25/98 | | | 0.018 | 32.6 | | |
| 1 | 22 | 1/26/98 | | | 0.000 | 32.0 | | |
| 2 | 22 | 1/26/98 | | | 0.000 | 31.1 | | |
| 3 | 22 | 1/26/98 | | | 0.010 | 30.7 | | |
| 1 | 23 | 1/27/98 | | | 0.000 | 30.8 | | |
| 2 | 23 | 1/27/98 | | | 0.000 | 30.1 | | |
| 3 | 23 | 1/27/98 | | | 0.000 | 30.1 | | |
| 1 | 29 | 2/2/98 | 183 | 1.00 | 0.840 | 29.1 | | 8.43 |
| 2 | 29 | 2/2/98 | 164 | 1.15 | 0.840 | 28.5 | | 8.43 |
| 3 | 29 | 2/2/98 | 143 | 1.05 | 0.940 | 28.5 | | 8.39 |
| 1 | 30 | 2/3/98 | | 0.994 | 0.904 | | | |
| 2 | 30 | 2/3/98 | | 1.08 | 0.780 | | | |
| 3 | 30 | 2/3/98 | | 1.02 | 0.957 | | | |
| 1 | 31 | 2/4/98 | | 1.00 | 0.002 | | | |
| 2 | 31 | 2/4/98 | | 1.00 | | | | |
| 3 | 31 | 2/4/98 | | 1.00 | | | | |

## APPENDIX F.

## MEDIA SCANS

Figure F-1. Scan of Media 1.

Figure F-2. Scan of Media 2.

Figure F-3. Scan of Media 3.

## VITA

Jonathan Thomas Scott is a Louisiana native currently residing on the bank of Bayou Manchac in Prairieville, Louisiana with his wife and son. In May, 1995 he was awarded the degree of Bachelor of Science in Biological Engineering, with a minor in environmental engineering, from Louisiana State University (LSU). He enrolled in the LSU Graduate School in 1996 and completed research on nitrification rates in a spouted-bed biological reactor in June, 1998.

Since completing his graduate coursework and research in 1998, he has worked as an environmental engineer for an environmental consulting firm and a plastics and inorganic chemicals manufacturing plant. He is currently employed as an environmental engineer at a specialty polyurethane chemicals manufacturing plant.

Scott, Jonathan Thomas, B.S., Louisiana State University, 1995
Master of Science in Biological and Agricultural Engineering, Fall Commencement, 2002
Major: Biological and Agricultural Engineering
Nitrification Rates in a Reversed-Flow, Spouted-Bed, Bioreactor Applied to Recirculating Aquaculture Systems
Thesis directed by Associate Professor Caye Drapcho
Pages in thesis, 159. Words in abstract, 312.

## ABSTRACT

The effects of media selection and organic loading on nitrification rates in a reversed-flow, three-phase, spouted-bed, bioreactor with draft-tube (A-1 Aquaculture Continuous-Cleaning Multifunctional Biofilter or CCMB) were studied. Experiments were conducted on three identical recirculating aquaculture systems (RAS) each having a CCMB unit with a unique plastic pelletized media and operated over five successive trials with varying components and operating conditions. Based upon organic loading, three of the five trials were grouped into two organic loading regimes of approximately 91 mg/L COD and 149 mg/L COD.

Comparing nitrification rates against the three media types by organic loading showed that media selection had a significant ($p<0.05$) impact on nitrification performance. At both levels of organic loading, the nitrification rates of Media 1 outperformed Media 2 and Media 3. The differences between the Media 1 rates and those of Media 2 and Media 3 were greater at the lower organic loading than at the higher loading, for nitratation than for nitritation, and for areal comparisons than for volumetric comparisons.

Comparing nitritation and nitratation rates against organic loading for each media type showed that organic loading had little impact on nitrification. A barely significant

($p<0.05$) difference between nitrification rates at the two organic loadings was observed only for Media 1 nitratation, where the nitratation rate was greater at the lower organic loading.

Throughout all five trials, the CCMB demonstrated the ability to successfully nitrify over organic levels ranging from 13.5 to 205.3 mg/L COD and without showing any signs of biofouling or other problems associated with traditional fixed-film nitrification systems. Media 1 achieved the highest mean nitrification rates during all trials with an average concentration-normalized volumetric nitritation rate of 223 g TAN/day-$m^3$ (0.0139 lb TAN/day-$ft^3$) and maximum of 254 g TAN/day-$m^3$ (0.0159 lb TAN/day-$ft^3$) observed during Trial 5, which had both the highest organic loading and flow rates of all trials.